VOLUME 77 *June 1958* NUMBER 6

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland, by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.
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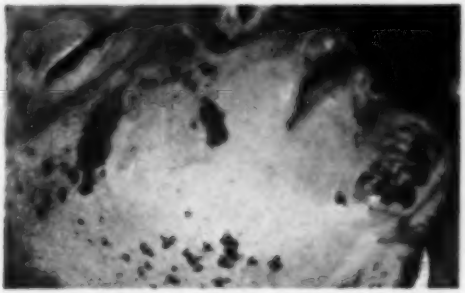

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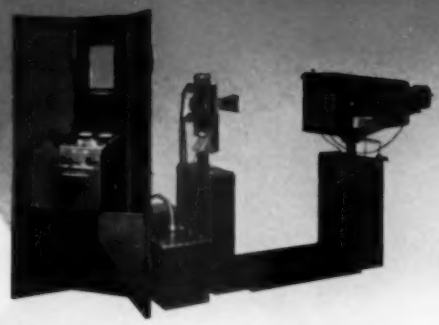

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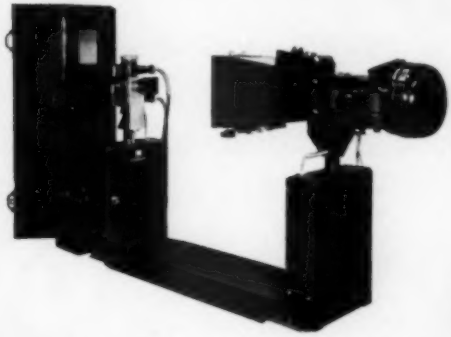

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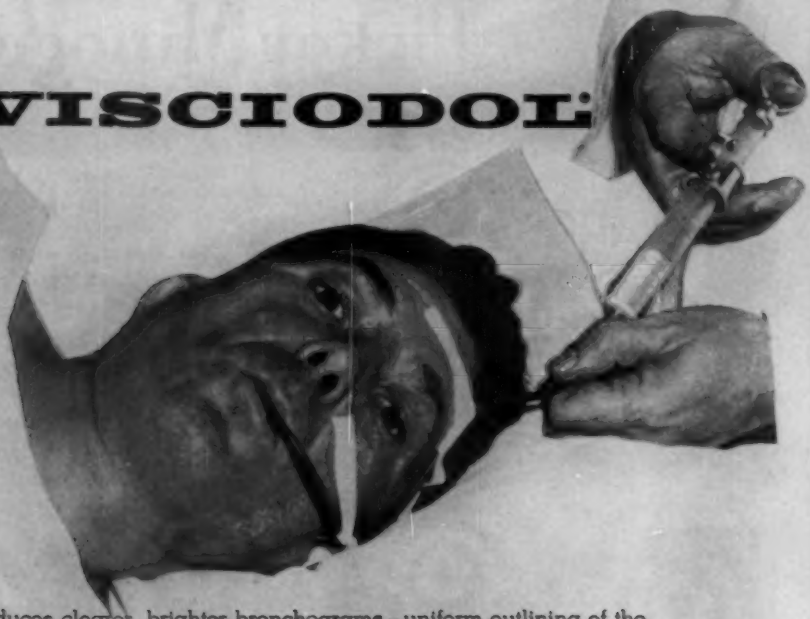

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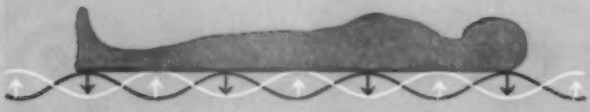

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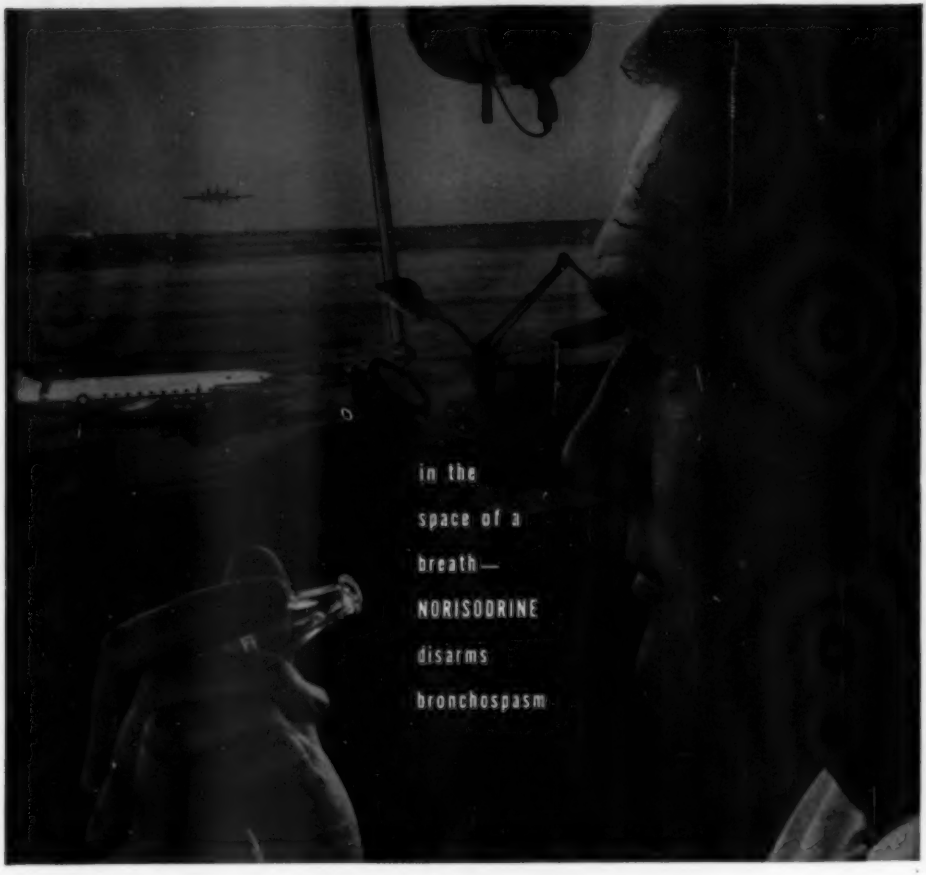

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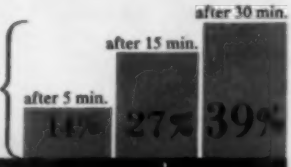

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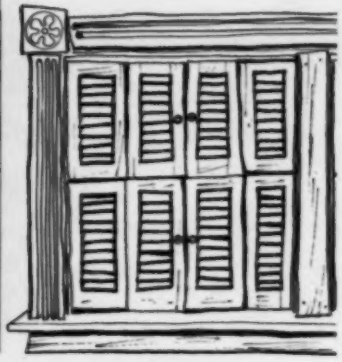

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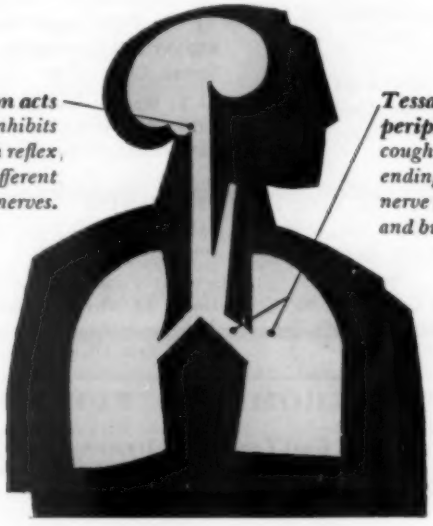

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1. Molthan, L., Cohen, R.V., and Zarafonetis, C.J.D.: Am. Rev. Tuber. *71*:220, 1955.
2. Cohen, R.V., Molthan, L., and Zarafonetis, C.J.D.: Dis. Chest *30*:418, 1956.

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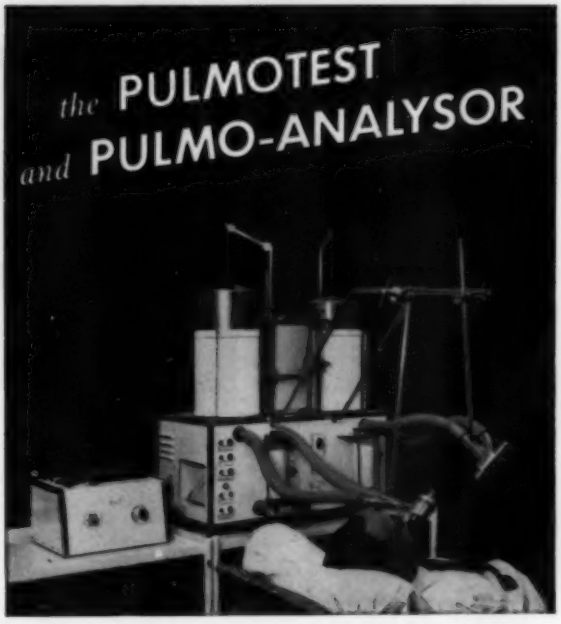

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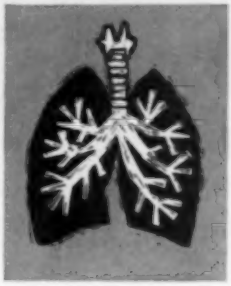

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 77 *June, 1958* NUMBER 6

COMMUNITY TRIALS OF BCG VACCINATION[1]

CARROLL E. PALMER, LAWRENCE W. SHAW, AND GEORGE W. COMSTOCK

(Received for publication March 7, 1958)

INTRODUCTION

Although BCG was first administered to a human subject in 1921 (1), its use during the next twenty-five years was based largely on results of laboratory studies and clinical experience gained from vaccinating highly exposed children and young adults. In the United States, little interest in BCG developed until the appearance in 1945–1946 of reports of three studies which embodied some of the features now considered essential for adequately controlled trials. Rosenthal, Blahd, and Leslie (2) and Aronson and Palmer (3) reported much less tuberculosis among vaccinated than unvaccinated subjects, whereas Levine and Sackett (4) could find no effect of vaccination on tuberculosis mortality. At about the same time, interest in BCG was further stimulated by the rapidly expanding Scandinavian programs which were soon to culminate in the international mass campaigns sponsored by UNICEF and the World Health Organization.

In spite of a growing enthusiasm for BCG, its use remained limited in some countries, including the United States and Great Britain, reflecting the judgment that convincing evidence of its value was still lacking. In 1947, G. S. Wilson of the British Medical Research Council found serious defects in the design and execution of all but one of the studies then reported, and concluded, "Though a presumptive case for the value of BCG vaccination has been established . . . the documentary evidence so far available is insufficient to allow any estimate to be made of the degree of protection it affords or even to prove conclusively that, in a civilized population, it has any protective effect at all. . . . A plea is entered for the carrying out of a properly controlled trial of BCG vaccination in Great Britain . . ." (5).

Meanwhile in the United States an advisory committee to the Surgeon General of the Public Health Service had expressed the view, in 1946, that the evidence was not sufficient to justify extensive use of BCG in this country and recommended that the Public Health Service undertake controlled trials in different

[1] From the Tuberculosis Program, Division of Special Health Services, Bureau of State Services, Public Health Service, Department of Health, Education, and Welfare, Washington 25, D. C.

kinds of populations (6). In line with these recommendations, several trials were planned by the Tuberculosis Program of the Public Health Service. They were to be strictly controlled trials, designed to avoid at least some of the deficiencies of earlier studies. A system of allocation was to be used which would ensure the similarity of a vaccinated and an unvaccinated control group, and every precaution would be taken to avoid bias. Tuberculin reactors, although not considered eligible for vaccination, would be included in the study population. By observing the number of new cases of tuberculosis appearing among the reactors, as well as among the vaccinated and controls, it would be possible to determine how much of the total amount of tuberculosis could have been prevented by vaccinating all nonreactors. The follow-up system would utilize medical, public health, and vital statistics reporting systems as an economical way to obtain sufficient and at the same time unbiased information on new cases of tuberculosis which develop in the study population. Large numbers of persons living under various conditions would be followed for a sufficient period of time to obtain information on both the immediate and long-range effects of vaccination.

After exploratory studies in 1947 among school children in Muscogee County, Georgia, and patients of mental hospitals in Georgia and Michigan, four controlled trials were undertaken. To test the value of BCG for selected "high-risk" groups, one trial was started among patients of Ohio mental institutions in 1948, and another among American Indian school children in 1949. A third trial was also started in 1949 among children in Puerto Rico; and, to test the usefulness of BCG in a general population in the continental United States, a fourth trial was begun in 1950 in Muscogee County, Georgia, and Russell County, Alabama.

The present paper is a progress report, intended only to summarize the results of the community trials in Puerto Rico and in Muscogee and Russell counties during the first six to seven years of follow-up observation. More detailed accounts will be presented in later papers.

Materials and Methods

The Trial in Puerto Rico

Puerto Rico, a tropical island in the West Indies, ranks among the most densely populated areas in the world. In 1950, approximately 2,200,000 persons lived on 3,400 square miles, an average of 650 persons per square mile. The population has increased rapidly—18 per cent from 1940 to 1950. According to the 1950 census, 80 per cent of the population was white, but racial classification is uncertain. Tuberculosis has been a serious problem in Puerto Rico for many years. Recorded mortality from the disease reached a peak in 1933 with a rate of 332 per 100,000 population. During the following fifteen years, the rate steadily declined to 179 in 1948; then more rapidly to reach a low of 33 in 1955. Throughout the entire period, tuberculosis mortality was much higher than in the continental United States, being six times higher in 1951 (7).

Planned as an island-wide mass program for children, the BCG trial in Puerto Rico was sponsored jointly by the Insular Departments of Health and Education, and the U. S. Public Health Service. Widespread publicity was given to the program, with extensive use of standard publicity media, and house-to-house visiting by schoolteachers and public health personnel. The tuberculin testing and vaccinating were carried out in the schools by teams of public health nurses who had been specially instructed and whose work was organized

and supervised by research personnel sent to the island by the U. S. Public Health Service. This phase of the program was started in September, 1949, and was terminated in May, 1951. By that time, the field teams had visited 1,433 schools in which 96 per cent of the school population was enrolled. An attempt was made to include the 40 per cent of the children of school age not enrolled in school by inviting them to participate at each school location. During the summer of 1950, and also during the following school year, special arrangements were made to include pre-school children. As in the international mass BCG campaigns, there was no roentgenographic screening of participants.

Unfortunately, the program was made a political issue and, as a result of adverse publicity directed against vaccination, large numbers of parents (and some of the children themselves) refused to have anything to do with the program or accepted only the tuberculin test. Nevertheless, as shown in table 1, nearly 200,000 of the million children on the island aged one through eighteen years participated in the program. Nearly 45 per cent of the children enrolled in school participated; among non-school children, participation was very poor.

Eligibility for vaccination was determined by the use of an intermediate dose of tuberculin, and nonreactors with no medical contraindications to vaccination were to be vaccinated or kept as controls according to their year of birth. In successive groups of three consecutive years, persons born in the middle year were allocated to the controls and not vaccinated; the others were to be vaccinated. It will be noted that this scheme should yield approximately twice as many vaccinees as controls and, in addition, should provide, for controls of a given birth year, a group of vaccinees of whom approximately half would be one year older and the other half one year younger than the controls. The method of allocation to vaccinee and control groups was revealed only to the few persons directly responsible for the technical operation of the program.

For purposes of this paper, the study population includes children aged one to eighteen years whose reactions to the intermediate dose of tuberculin were known. The age distribution and tuberculin status of the study population are shown in figure 1 and table 2. Approximately 82,000, or two-fifths of the population, were classified as tuberculin reactors and therefore were ineligible for vaccination. The nonreactors, totaling nearly 110,000, consist of "refusals," controls, and vaccinees. The large group who refused vaccination (more than 30,000 children) are included so that a correct estimate can be made of the amount of tuberculosis arising among all nonreactors.

The proportion of reactors increased markedly with age. For example, 21 per cent were reactors at age six and 69 per cent at age eighteen. This pronounced increase with age may be taken as some indication of the magnitude of the tuberculosis problem in Puerto Rico during the lifetime of these children, although the percentages are undoubtedly inflated because of the high prevalence of nonspecific tuberculin sensitivity in the island population.

TABLE 1

SIZE OF STUDY POPULATION IN RELATION TO ISLAND POPULATION, BY AGE, PUERTO RICAN TRIAL

Age Group	Island Population (1951)	Study Population	
		Number	Per Cent of Island Population
(years)			
Total	1,088,600	191,827	17.6
1–6	446,200	25,103	5.6
7–12	357,900	95,114	26.6
13–18	284,500	71,610	25.2

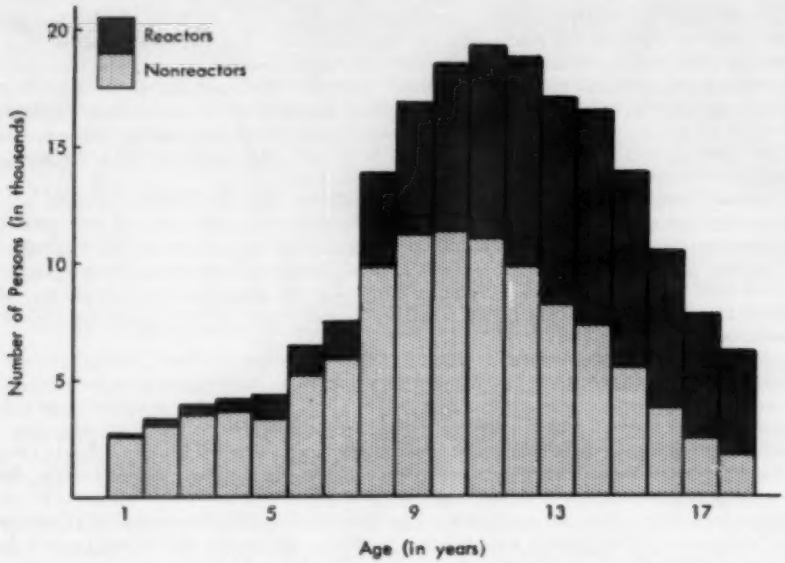

FIG. 1. Distribution of Puerto Rican study population by age and tuberculin status (10 TU) at entry to the trial.

TABLE 2

COMPOSITION OF STUDY POPULATION BY TUBERCULIN AND VACCINATION STATUS AND AGE, PUERTO RICAN TRIAL

Age Group	Total Persons	Tuberculin Reactors	Tuberculin Nonreactors		
			Refusals	Controls	Vaccinees
Total	191,827	82,269	31,586	27,338	50,634
1–6	25,103	3,906	2,144	5,976	13,077
7–12	95,114	35,869	20,269	14,215	24,761
13–18	71,610	42,494	9,173	7,147	12,796

The Trial in Muscogee and Russell Counties

Muscogee County, Georgia, and Russell County, Alabama, are adjacent counties located near the middle of the common boundary of the two states. Their combined population was about 155,000 in 1950 and almost 200,000 in 1956. One-quarter of the population lived in rural areas; most of the remainder, in the cities of Columbus (Georgia) and Phenix City (Alabama). Nearly one-third of the total population was Negro. The average annual tuberculosis mortality rate for the years 1949–1951 was 25 per 100,000 which, when adjusted for racial composition, was slightly lower than the corresponding rate for the United States as a whole (8). The principal reason for selecting this community for a BCG trial was that the Tuberculosis Program of the Public Health Service had established a field research station in the Health Department of Muscogee County in 1946 (9). Clinical, statistical, and epidemiologic facilities required for the trial were thus already available in the community.

The BCG trial was a cooperative enterprise, conducted by the Public Health Service and the two county health departments, with assistance from the two state health departments. In the spring of 1950, all persons more than five years old were urged to participate in a program which offered 70 mm. photofluorographic examinations, tuberculin tests and, when indicated, BCG vaccination. The approach to the community was generally similar to that of mass roentgenographic surveys being carried out in many other communities at the time (10). Various media of mass communication provided extensive publicity, speakers were furnished for group meetings and, in most places in the two counties, door-to-door canvassing by volunteer workers was done to stimulate participation and to ensure that all residents knew about the program.

The tuberculin tests and photofluorographic examinations to determine eligibility for vaccination were performed by Public Health Service teams who set up field clinics in schools, industries, and community centers. In contrast to the Puerto Rican experience, there was no opposition to the program. The pattern of response was similar to that reported from many mass roentgenographic surveys in this country (11): about half the population more than five years of age participated. As may be seen from table 3, participation was best among the school age group, with a progressive decrease with advancing age. Negroes participated somewhat more fully than whites. The few children younger than five years of age who were brought for examination have been included in the study.

The study population, shown in figure 2 and table 4, was restricted to residents of the two counties whose photofluorograms were classified by two film readers as negative for

TABLE 3

SIZE OF STUDY POPULATION IN RELATION TO CENSUS POPULATION, BY AGE, MUSCOGEE-RUSSELL TRIAL

Age Group	Census Population (1950)	Study Population	
		Number	Per Cent
Total			
(years)			
Total	137,503	64,136*	46.4
5–19	42,034	26,542	63.1
20–39	56,869	24,174	42.5
40+	38,600	13,091	33.9
White			
Total	92,704	39,589*	42.5
5–19	26,657	15,906	59.7
20–39	39,957	15,076	37.7
40+	26,090	8,388	32.2
Negro			
Total	44,790	24,547*	54.5
5–19	15,377	10,636	69.2
20–39	16,912	9,098	53.8
40+	12,510	4,703	37.6

* These totals include persons less than five years old (219 white, 110 Negro).

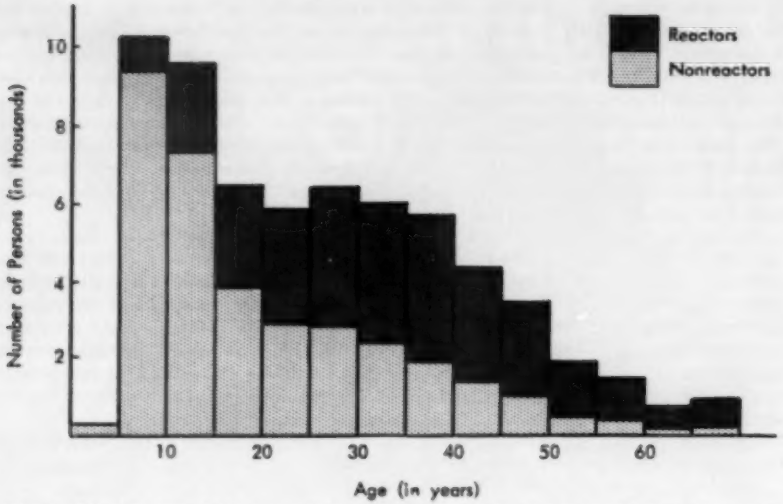

FIG. 2. Distribution of Muscogee-Russell study population by age and tuberculin status (5 TU) at entry to the trial.

TABLE 4

COMPOSITION OF STUDY POPULATION BY TUBERCULIN AND VACCINATION STATUS, RACE AND AGE, MUSCOGEE-RUSSELL TRIAL

Age Group	Total Persons	Tuberculin Reactors	Tuberculin Nonreactors	
			Controls	Vaccinees
Both Races				
Total	64,136	29,369	17,854	16,913
White				
Total	39,589	15,099	12,702	11,788
5–19*	16,125	2,365	7,101	6,659
20–39	15,076	7,361	3,949	3,766
40+	8,388	5,373	1,652	1,363
Negro				
Total	24,547	14,270	5,152	5,125
5–19*	10,746	3,644	3,493	3,609
20–39	9,098	6,665	1,247	1,186
40+	4,703	3,961	412	330

* Includes persons less than five years old.

pulmonary abnormalities, who had no obvious medical contraindications to vaccination, and whose tuberculin reactions were read at seventy-two hours. While the prevalence of tuberculin reactors among young persons was much lower than in Puerto Rico, the inclusion of substantial numbers of persons more than twenty years of age resulted in the reactors' forming nearly half of the study population. Nonreactors to tuberculin were assigned to the vaccinee or control group according to their year of birth. Persons with alternate birth years were vaccinated; the others were left unvaccinated as controls. As in Puerto Rico, the method of allocation to control and vaccinee groups was not divulged. Persons who came for the tuberculin test and photofluorograms but refused vaccination have been removed from both control and vaccinee groups—they are not included in the study population for the present report because their numbers were too few to make a significant difference in the results.

The proportion of tuberculin reactors increased with age and was greater among Negroes than whites. At twenty years of age, 35 per cent of the whites and 60 per cent of the Negroes were classified as reactors; at forty years of age, 59 per cent of the whites and 83 per cent of the Negroes. As in Puerto Rico, the frequency of reactors is undoubtedly inflated by the high prevalence of nonspecific tuberculin sensitivity in the area (12–14).

Tuberculin Testing and Vaccination Procedures

The tuberculin used in both trials was PPD (RT 19-20-21) obtained from the State Serum Institute in Copenhagen, Denmark. A central laboratory for each trial supplied freshly prepared dilutions to the testing teams in the field. The tests were given by intracutaneous injection of 0.1 ml. in the volar surface of the forearm. The reactions were read by carefully measuring the diameter of induration on the third day after injection. So that assignment to the control or vaccinee group would not be influenced by subjective factors, the readers of the tuberculin reactions were not allowed to know the birth year of the subjects or the results of any other examination. In the Muscogee-Russell trial, in which chest photofluorograms were used to screen the study population, the film readers were also denied access to any other information concerning the subject.

In the schools in Puerto Rico, a two-dose series of tuberculin tests was used to select children for vaccination. The first dose contained 1 tuberculin unit (1 TU = 0.00002 mg.) of PPD; the second dose, given to nonreactors to the first test, contained 10 TU. Pre-school children were given only the 10 TU test in making the selection for vaccination. Children with 6 or more mm. of induration to either the 1 TU or 10 TU test were classified as reactors; those with reactions of less than 6 mm. to 10 TU were classified as nonreactors. Vaccinees and controls, but not those who refused vaccination, were tested also with 100 TU. By giving this test only to nonreactors who signified their willingness to accept vaccination, but not to those who refused, the act of vaccination was made less conspicious, thereby helping to maintain similarity in the treatment of vaccinees and controls at the time the former were being vaccinated. The principal purpose of giving the 100 TU test was, however, to provide information for studying the relationship between low-grade tuberculin sensitivity and resistance to tuberculosis, and whether such naturally acquired sensitivity affects the results of vaccination. In the Muscogee-Russell trial, only a single test dose of 5 TU was used. Reactors were defined as persons with 5 or more mm. of induration; nonreactors as those with smaller reactions.

Source of vaccine: The vaccine for the Puerto Rican trial was prepared by Dr. K. Birkhaug at the New York State Department of Health, and was shipped by air in refrigerated containers. It was used on the third or fifth day after preparation. A dose of 0.1 mg. of BCG organisms in 0.1 ml. of diluent was given intracutaneously over the insertion of the left deltoid muscle. For the Muscogee-Russell trial, fresh BCG vaccine was obtained from Dr. S. R. Rosenthal of the Research Foundation in Chicago. It was also shipped by air and kept refrigerated until its use within five days of preparation. Vaccination was performed by Rosenthal's method of multiple tangential acupuncture on the deltoid area of the left arm. In both trials, the vaccinators were specially trained nurses.

Post-vaccinal tuberculin sensitivity: As a precaution against drawing attention to differences between vaccinees and controls, no systematic follow-up examinations were made of the local vaccinal lesions. For the same reasons, all studies of post-vaccinal tuberculin sensitivity were made on samples which always included other population groups in addition to vaccinees. The results of these studies will be discussed in detail in a later paper, but it may be mentioned here that in Puerto Rico 89 per cent of the vaccinees had reactions of 6 or more mm. to 10 TU one to two years after vaccination; and in the Muscogee-Russell area 54 per cent had reactions of 5 or more mm. to 5 TU two years after vaccination.

Follow-up Procedures

In planning the follow-up phase of the trials, the decision was made to use only the established medical, public health, and vital statistics reporting systems for identifying new cases of tuberculosis appearing in the study populations during the years following the vaccination programs. This decision reflected the opinion that, with adequate safeguards, the established systems would provide unbiased data and be sufficiently complete and accurate to bring out any significant effects of vaccination on the study populations. Moreover, follow-up observation of several hundred thousand study participants for a long period of time was economically feasible by utilizing the existing systems. A method of follow-up based on systematic, periodic examinations of the study populations was never seriously considered. While such examinations might have identified more persons with abnormal chest findings, it was not likely that the number of cases of clinically significant tuberculosis detected in either study population would have been increased materially. Of greater importance, however, was the fear that it would be extremely difficult, perhaps impossible, to avoid bias in the diagnosis of cases if periodic follow-up examinations were recognized as part of the vaccination program.

As safeguards against the introduction of bias in the established reporting systems, certain policies and techniques were adopted. Thus, care was taken to avoid any official statement or act which would publicize the fact that the vaccination programs were in reality controlled trials. The initial testing and vaccination phases of both programs were offered as limited projects of the mass survey type. In Muscogee and Russell counties the whole population–and in Puerto Rico the child population–were simply urged to come for the examination, and vaccination if indicated. No mention was made of a need for follow-up examinations of any kind. Information on the initial tuberculin and vaccination status of study participants was not divulged so that medical decisions to record any individual as suspected of having tuberculosis had to be made independently of that information. In brief, deliberate effort was made to play down the study aspects of the program in order to avoid creating any interest in the tuberculin and vaccination status of the study participants which might influence the subsequent discovery or diagnosis of tuberculosis.

Individual field record cards containing identifying information and results of the testing and vaccination were prepared for all persons participating in the programs. In each study area these cards were assembled into a roster, against which official reports of cases of tuberculosis and suspects and certificates for all deaths were currently matched. To ensure that no cases known to official agencies were overlooked, reports of tuberculosis hospital admissions and discharges and lists of patients furnished antituberculous drugs were also matched against the rosters. Care was taken to ensure accurate and unbiased matching of reports, and various tests were devised to check the matching procedures. In instances in which uncertainties arose in matching a report, field investigations were made to verify the accuracy of the identifying data.

The definition of what would be accepted for present purposes as a case of tuberculosis differed somewhat in the two trials. In Puerto Rico, any person reported as having tuberculosis or as dying of the disease was accepted as a case. The vast majority of these reports were made by the Insular Health Department's twenty tuberculosis centers distributed throughout the island. In line with the policy of not disturbing the existing diagnostic procedures in any way, no attempt was made to verify the accuracy of the diagnosis on any

case or death report, even though errors undoubtedly occurred. Owing to the complete separation of the study roster from other medical and public health facilities, it seems very unlikely that the diagnosis and reporting of cases and deaths in Puerto Rico could have been influenced in any way by the initial tuberculin and vaccination status of the study participants.

In the Muscogee-Russell trial the diagnosis and reporting of newly appearing cases of tuberculosis or deaths from the disease are thought to be as accurate and complete as can be obtained for the general population of a community. Since 1946 one of the authors (GWC) has personally supervised an energetic campaign to find all old and new cases of tuberculosis, nearly all of which were brought under observation in the health departments' clinics. Cooperation by the medical profession and community agencies in referring cases or suspected cases has remained unusually good, and justifies the view that few known cases of active disease are unknown to the health departments. However, since the physician who served as the tuberculosis control officer for the two counties was also the local medical director of the vaccination trial, as well as other tuberculosis research studies, it is difficult to be certain that the diagnosis of new cases was always made without knowledge of their status at entry to the trial. Consequently, it was necessary to obtain an independent review of the cases. It was not sufficient merely to assemble the cases and suspects as finally diagnosed by the chest clinics. Rather, the films for all persons ever suspected of having tuberculosis were reviewed. When the suspicion was first raised, the status of the individual in the trial was not known. Indeed, even the fact of his participation in the trial was then unknown. For these persons, all films, whether they were finally interpreted as tuberculous or nontuberculous by the clinics, were submitted to a reviewer who had never been associated with the study and who was told only that the films were of persons examined in a chest clinic. The results of this independent review gave no indication of any bias in the recognition or classification of new cases of tuberculosis.

The follow-up period for the trial in Puerto Rico extends to January 1, 1957, a period of 6.3 years from the mid-point of the vaccination phase of the trial. For Muscogee and Russell counties, the follow-up extends to April 1, 1957, a period of 7 years. Although some of the persons who entered the studies moved away during the follow-up period and were lost to observation, it has not been considered necessary for the purposes of this summary report to take account of this fact. Tuberculosis case rates for comparing the various subgroups are based on the number of persons who entered the study. So far as can be judged, case-finding activities and diagnostic criteria have remained uniform throughout the period of observation.

Comparison of Controls and Vaccinees

The allocation, according to year of birth, of nonreactors to control and vaccinee groups could be expected to produce two groups at equal risk of developing tuberculosis during the ensuing years. However, the practical field operations must be examined in detail to determine whether any violation of the allocation scheme interfered with the expected similarity of the two groups. As a further check on that similarity it is also necessary to compare the vaccinees and controls with respect to age, race, sex, and other characteristics. In the present paper only the principal complications that arose in the field work will be mentioned and the customary comparisons made between control and vaccinee groups.

The most obvious item to be considered is the number of persons in the control and vaccinee groups. If there had been equal numbers of eligible participants for every birth year, there should be twice as many vaccinees as controls in the Puerto Rican trial, and the same number of vaccinees and controls in the Muscogee-Russell trial. However, in table 5, it may be seen that there are more controls in each trial than would be expected. A number of factors are responsible for the difference between the observed and expected numbers, one of them being that the number of eligibles varied systematically with birth year, owing to the changing pattern of participation and the increasing prevalence of tuberculin sensitivity with age. More important problems arose because the controls were not given an

TABLE 5

PERCENTAGE DISTRIBUTION OF THE VACCINATED AND CONTROL GROUPS, BY RACE, SEX, AGE, RESIDENCE, AND INITIAL TUBERCULIN SENSITIVITY

	Puerto Rican Trial			Muscogee-Russell Trial		
	Characteristic	Controls	Vaccinees	Characteristic	Controls	Vaccinees
Race	White	88.5	88.8	White	71.1	69.7
	Non-white	11.5	11.2	Non-white	28.9	30.3
Sex	Male	47.6	48.0	Male	40.6	40.3
	Female	52.4	52.0	Female	59.4	59.7
Age	1–6	21.9	25.8	5–19	59.3	60.7
	7–12	52.0	48.9	20–39	29.1	29.3
	13–18	26.1	25.3	40 and older	11.6	10.0
Residence	Urban	38.6	36.6	Muscogee	73.2	72.5
	Rural	61.4	63.4	Russell	26.8	27.5
Initial tuberculin sensitivity	0–5 mm., 100 TU	68.6	71.2	0 mm., 5 TU	47.3	47.5
	6+ mm., 100 TU	31.4	28.8	1–4 mm., 5 TU	52.7	52.5
Number of persons		27,338	50,634		17,854	16,913

adequate placebo vaccination, a failing shared by all other trials of BCG. Each additional procedure to which any study group or segment of it is subjected usually results in a further loss in the number of participants. Thus, some subjects did not refuse vaccination until they were confronted with the vaccinating needle. As the controls did not have this experience, it was not possible to identify the comparable group among them who would also have refused at that point. Similarly, exclusions for medical contraindications were less frequent among controls than among candidates for vaccination. Each control group is therefore somewhat larger than it otherwise would have been. Cold though the comfort may be, other controlled trials have also failed to achieve the expected number of controls and vaccinees, presumably because of similar difficulties (2–4, 15, 16).

The results of several comparisons of controls and vaccinees are summarized in table 5. In addition to race, sex, and age composition, the two groups are compared with respect to urban and rural residence in Puerto Rico and county of residence in the Muscogee-Russell area. Comparisons are also made of the prevalence of low-grade tuberculin sensitivity, as judged by reactions to the 100 TU test in Puerto Rico and by small reactions to the 5 TU test in the Muscogee-Russell area. It is evident that the controls and vaccinees in each study population are similar; after careful consideration of the possible effects of the slight differences between the groups, it is believed that they could not alter the conclusions drawn from the present results.

RESULTS

In evaluating the usefulness of BCG vaccination for any population, it is important to remember that only those who have not had a tuberculous infection—the nonreactors to tuberculin—are ordinarily considered eligible for vaccination and for any direct advantages that may accrue from vaccination. But to measure the usefulness of vaccination only in terms of its value to non-

TABLE 6

NUMBER AND PER CENT OF CASES OF TUBERCULOSIS AMONG REACTORS AND NONREACTORS (WITH EFFECT OF VACCINATION REMOVED)

Tuberculin Status on Entry	Puerto Rican Trial		Muscogee-Russell Trial	
	Number of Cases	Per Cent	Number of Cases	Per Cent
Total	1,149	100.0	216	100.0
Reactors	856	74.5	161	74.5
Nonreactors	293*	25.5	55*	25.5

* Estimated as explained in text.

reactors is to ignore the fact that they may or may not contribute significantly to the total amount of tuberculosis that would occur over a period of time in the whole population. Obviously the value of vaccination for existing populations depends on two factors: the proportion of cases in the total population destined to arise among the nonreactors and the effectiveness of vaccination in preventing those cases. The point may be illustrated by two examples. At one extreme, BCG or any immunizing procedure could be potentially very useful in a population composed almost entirely of nonreactors who would be subjected to a high risk of tuberculosis during the coming years. At the other extreme, vaccination—regardless of its effectiveness among nonreactors—could have practically no value in a population composed almost entirely of tuberculin reactors.

In view of these considerations, the results of the trials in Puerto Rico and in Muscogee and Russell counties will be presented in the form of answers to the following three questions:

1. What proportion of the total number of cases of tuberculosis in each study population was subject to the direct immunizing effects of vaccination?
2. How effective was vaccination among those who were vaccinated?
3. What reduction in the total number of cases of tuberculosis could have been accomplished by vaccinating all nonreactors?

Tuberculosis among reactors and nonreactors:[2] To answer the first question it is necessary to know the number of cases of tuberculosis that developed among reactors and to estimate the number that would have developed among nonreactors had none of them been vaccinated.

In the Puerto Rican trial, 1,149 cases of tuberculosis would have been expected without vaccination—856 in the tuberculin reactors and 293 in the nonreactors, as shown in table 6. The 856 cases shown for the reactors were those actually reported for that group. The 293 cases shown for the nonreactors have been esti-

[2] The definitions of reactors and nonreactors should be kept clearly in mind throughout this paper, and particularly in the present section. Both terms refer to the classification of the study population at entry to the trials, and not to their subsequent status. Thus while most cases among the nonreactors could be expected to react to tuberculin at the time of diagnosis, they are still referred to as "nonreactors," their status when first tested in these trials.

mated in the following way. The number of cases expected among vaccinees if they had not been vaccinated is obtained by applying the case rate among controls to the vaccinated group; the result is 135 cases. These 135 expected cases among vaccinees added to the 73 cases actually reported among the controls and the 85 reported among the refusals gives the total number of cases, 293, that would have occurred among nonreactors if no one had been vaccinated. Thus, of the total amount of tuberculosis expected in the Puerto Rican study population, 293, or 25 per cent, of the 1,149 potential cases would have been subject to the direct effects of vaccination.

In the Muscogee-Russell trial, the findings were similar although based on a smaller study population and much smaller numbers of cases. The total tuberculosis problem is represented by 216 cases, 161 of which were reported among the tuberculin reactors. The remaining 55 cases, 25 per cent of the total, represent those subject to the effects of vaccination.

An important consideration is whether or not the proportion of cases emerging from reactors and nonreactors changed during the observation period. As shown in the lower section of table 7, the proportion has remained remarkably constant throughout the seven years in both study areas.

The number of cases among the reactors might have been expected to decline, at least during the first few years, because the group must have included some

TABLE 7

NUMBER AND PER CENT OF CASES OF TUBERCULOSIS AMONG REACTORS AND NONREACTORS,* BY YEAR OF OBSERVATION

Year of Observation	Puerto Rican Trial		Muscogee-Russell Trial	
	Reactors	Nonreactors*	Reactors	Nonreactors*
Number of Cases				
Total	856	293	161	55
First	133	24	18	2
Second	139	62	20	10
Third	137	45	19	6
Fourth	134	48	23	10
Fifth	148	55	28	17
Sixth	104†	37†	26	2
Seventh	61†	22†	27	8
Percentage of Cases				
Total	74.5	25.5	74.5	25.5
First–second	76.0	24.0	76.0	24.0
Third–fourth	74.5	25.5	72.4	27.6
Fifth–seventh	73.3	26.7	75.0	25.0

* With effect of vaccination removed, i.e., estimated in the same manner as for table 6.
† Based on incomplete years of observation.

recent convertors, and morbidity is well known to be high in the first few years after infection. However, as is shown in the upper part of table 7 and in figure 3, there is no evidence of a decline in either study area. In fact, in the Muscogee-Russell trial, the numbers appeared to be increasing somewhat during the early years, a finding that may be related to the fact that a number of cases had been removed from the reactor group in that trial by the roentgenographic screening, and it may have taken several years for the emergence of new cases to build up

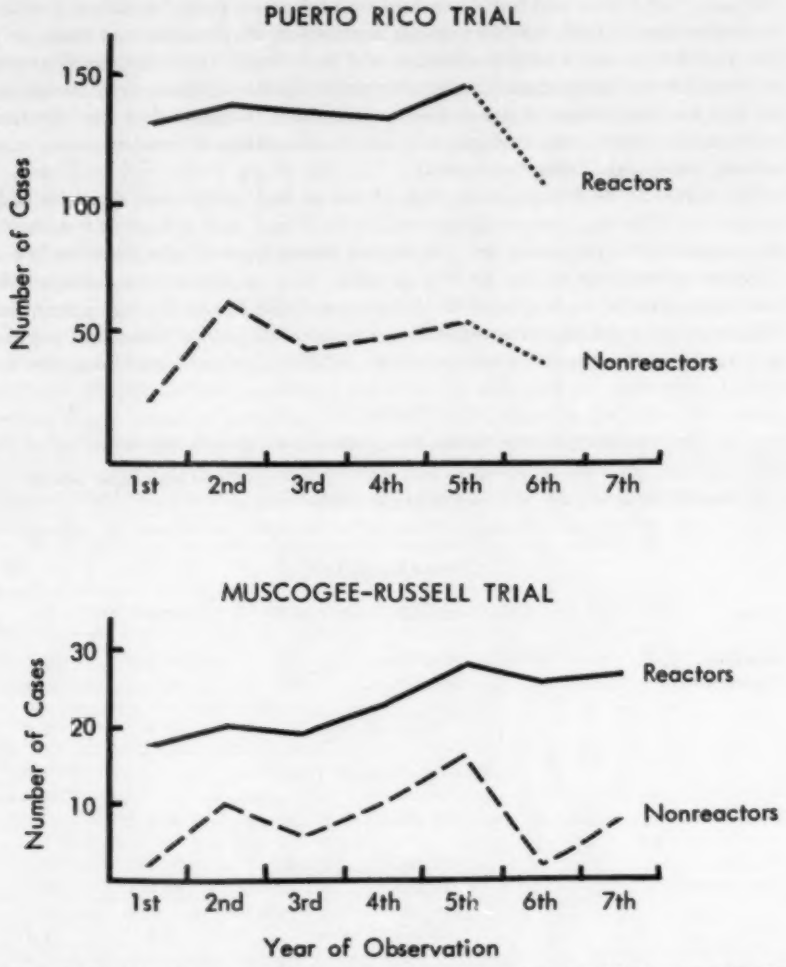

FIG. 3. Number of cases per year among reactors and nonreactors. (Sixth-year values for Puerto Rico are estimated from 95 per cent complete data.)

to a "normal" level. The absence of a decrease in the annual number of cases from the reactors implies that there were relatively few recent convertors among them and this, in turn, points to low infection rates during the years immediately preceding the trials.

Among the nonreactors, the number of cases might have been expected to increase substantially during the observation period because the number of infected persons among them would progressively increase. But that expectation is not borne out by the results. For Puerto Rico, there was a small number the first year, but higher and fairly constant numbers each year thereafter. For the Muscogee-Russell trial, the cases among nonreactors were too few and irregularly distributed to permit a reliable estimate of a time trend. The failure to observe a clear-cut increase throughout the six-year period in Puerto Rico, even though no account has been taken of losses due to emigration, suggests that the infection rates among nonreactors, and possibly also the incidence of disease among convertors, decreased during that period.

The answer to the first question, then, is that in both study areas approximately one-quarter of the total amount of tuberculosis could have been influenced by vaccination; moreover, the proportion has not changed during a period of six to seven years.

Before proceeding to the second question, it is of interest to consider the incidence rates for each group. As is shown in table 8, the average annual incidence of cases of tuberculosis in Puerto Rico was 163 per 100,000 initial population for the reactors and 43 for nonreactors; in Muscogee and Russell counties the

TABLE 8

INCIDENCE OF TUBERCULOSIS AMONG REACTORS AND NONREACTORS*

Tuberculin Status on Entry	Study Population	Number of Cases	Average Annual Incidence per 100,000	
			Crude	Adjusted†
Puerto Rican Trial				
Total	191,827	1,149	95.3	—
Reactors	82,269	856	163.3	165.8
Nonreactors	109,558	293*	43.1	46.9
	Ratio of rates		3.8	3.5
Muscogee-Russell Trial				
Total	64,136	216	48.2	—
Reactors	29,369	161	78.3	67.1
Nonreactors	34,767	55*	22.4	31.6
	Ratio of rates		3.5	2.1

* With effect of vaccination removed.

† Adjusted to the race-sex-age composition of the total study population of each trial.

rates were much lower, being 78 and 22, respectively. In both areas, the risk of developing tuberculosis was more than three times greater for reactors than for nonreactors. Part of this disproportionate risk might be related to the composition of the populations, for in both trials the reactors contained a larger proportion of older subjects, of males, and of non-whites than the nonreactors. Because of these differences, the rates were adjusted to the race-sex-age composition of the total study population in each trial. As may be seen in the table, even after allowing for differences in the composition of the two groups, the risk of tuberculosis was much greater for reactors than for nonreactors.

Another consideration relative to incidence rates is the effect of the preliminary roentgenographic screening in the Muscogee-Russell trial. As a result of this procedure, a substantial number of cases of tuberculosis and suspected tuberculosis were removed from the study population. Since the great majority of the removed cases were reactors, it is evident that, had the screening not been done, the ratio of the rates for reactors and nonreactors would have been greater. Similarly, the proportion of cases emerging from reactors would also have been greater.

Tuberculosis among controls and vaccinees: The question on the effectiveness of vaccination among those who were vaccinated can be answered directly by comparing the tuberculosis experience of the control and vaccinee groups, as is summarized in table 9. In Puerto Rico, 73 cases were reported among 27,338 controls and 93 cases among 50,634 vaccinees, an average annual incidence of 43.2 per 100,000 initial population for controls and 29.8 for vaccinees. In this instance, age adjustment results in virtually no change in the rates, indicating that the minor differences in age composition between the two groups did not influence their tuberculosis experience. The difference between the rates for controls and vaccinees is statistically significant—the observed difference or a larger one (favorable to vaccination) could be expected to arise by chance only

TABLE 9

INCIDENCE OF TUBERCULOSIS AMONG CONTROLS AND VACCINEES

Study Category	Study Population	Number of Cases	Average Annual Incidence per 100,000
Puerto Rican Trial			
Controls	27,338	73	43.2
Vaccinees	50,634	93	29.8
Percentage reduction in tuberculosis among vaccinees—30.9			
Muscogee-Russell Trial			
Controls	17,854	28	22.4
Vaccinees	16,913	17	14.4
Percentage reduction in tuberculosis among vaccinees—35.9			

TABLE 10

CASES OF TUBERCULOSIS AMONG CONTROLS AND VACCINEES BY YEAR OF OBSERVATION

Year of Observation	Puerto Rican Trial		Muscogee-Russell Trial	
	Controls	Vaccinees	Controls	Vaccinees
Total	73	93	28	17
First	6	14	1	0
Second	17	13	5	3
Third	13	16	3	3
Fourth	11	17	5	1
Fifth	13	18	9	4
Sixth	8*	10*	1	4
Seventh	5*	5*	4	2

* Based on incomplete years of observation.

once in 100 times. *The reduction in tuberculosis attributable to vaccination is 31 per cent, with 90 per cent confidence limits of 10 and 47 per cent.*

In the Muscogee-Russell trial, there were 28 cases among 17,854 controls and 17 cases among 16,913 vaccinees, an average annual incidence of 22.4 and 14.4 per 100,000, respectively. *The apparent reduction in tuberculosis attributable to vaccination is 36 per cent, but the difference between the rates could readily have arisen by chance.*

Because the effectiveness of vaccination is considerably less than has been reported in some other studies, it is especially pertinent to consider whether the estimate of effectiveness changed during the course of the trials. The cases among vaccinee and control groups by year of observation are shown in table 10. It is apparent, particularly for the Muscogee-Russell trial, that the numbers of cases appearing each year in each group are small, with no readily perceptible trend with time. In Puerto Rico, at the end of two years 23 cases had been reported for the controls, and 27 for the vaccinated. At that point, the vaccine appeared to be 37 per cent effective. During the next two years the results were somewhat less favorable, so that at the end of four years the apparent effectiveness had declined to 31 per cent. The percentage did not change during the remainder of the observation period. For the Muscogee-Russell trial the findings hardly warrant analysis by time, but the apparent effectiveness at the end of four years was 47 per cent as compared with 36 per cent at the end of seven years.

Potential usefulness of BCG vaccination: Based on the foregoing results, it is now possible to estimate what reduction in the total number of cases of tuberculosis could have been accomplished by vaccinating all of the nonreactors. The findings of these trials show that the potential usefulness of BCG was not very great, as may be seen from the material summarized in table 11 and illustrated for Puerto Rico in figure 4. The complete circle in the diagram represents the total tuberculosis that would have emerged if BCG had not been used; this amounts to 1,149 cases, as previously shown (table 6). The large dark section of the figure indicates that three-fourths of the cases were reported among persons who were

TABLE 11

RECAPITULATION OF THE POTENTIAL USEFULNESS OF BCG

	Puerto Rican Trial	Muscogee-Russell Trial
A. Observed cases:		
Total	1,107	206
Reactors	856	161
Nonreactors	251	45
B. Tuberculosis problem: estimated cases if NO nonreactors had been vaccinated:		
Total	1,149	216
Reactors	856	161
Nonreactors	293	55
C. Estimated cases if ALL nonreactors had been vaccinated:		
Total	1,058	196
Reactors	856	161
Nonreactors	202	35
D. Cases that could have been prevented by BCG if all nonreactors had been vaccinated:		
Number	91	20
Per cent of tuberculosis problem	7.9	9.3

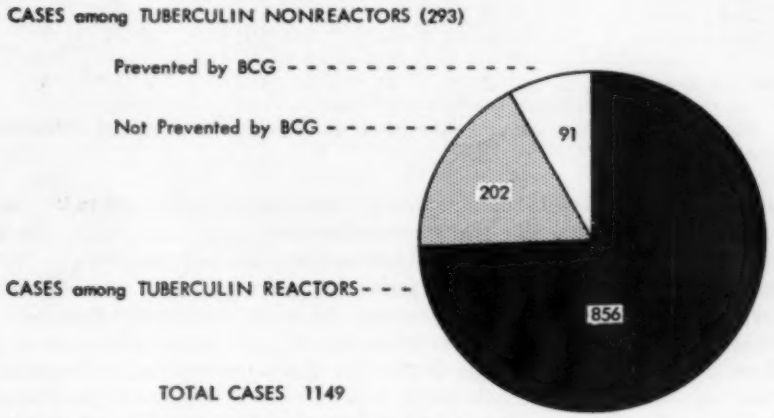

FIG. 4. Diagram showing the potential usefulness of BCG vaccination for the total study population in Puerto Rico.

reactors on entry to the trial, leaving one-fourth, or 293 cases, which could have been directly affected by BCG. *However, as BCG was 31 per cent effective, its administration to all nonreactors in the study population, had they all been willing to be vaccinated, would have prevented 91 cases, or 7.9 car cent, of the total of 1,149 expected.*

Except for the much smaller numbers of cases involved, the findings in the Muscogee-Russell trial were almost identical with those for Puerto Rico. In this instance, 216 cases would have been expected without vaccination, 55 of them among nonreactors; and, of these, 20 might have been prevented had all non-

TABLE 12

CLASSIFICATION OF CASES, BY TUBERCULIN AND VACCINATION STATUS, PUERTO RICAN TRIAL

Tuberculosis Classification	Tuberculin and Vaccination Status				
	Total	Reactors	Refusals	Controls	Vaccinees
Total	1,107 (63)	856 (50)	85 (6)	73 (5)	93 (2)
Pulmonary	1,006 (44)	784 (36)	74 (3)	65 (3)	83 (2)
Far advanced	238 (44)	196 (36)	13 (3)	14 (3)	15 (2)
Moderately advanced	329	267	27	16	19
Minimal	315	252	22	18	23
Primary	116	63	12	17	24
Stage unknown	8	6	0	0	2
Nonpulmonary	76 (17)	57 (13)	7 (2)	7 (2)	5
Miliary, meningeal	19 (13)	15 (11)	1	2 (2)	1
Pleural	4	2	0	2	0
Lymph node	37 (3)	29 (2)	4 (1)	3	1
Bone and joint	16 (1)	11	2 (1)	0	3
Not specified	25 (2)	15 (1)	4 (1)	1	5

Figures in parentheses indicate tuberculosis deaths, which are included in the case count.

reactors been vaccinated. This amounts to a reduction of 9.3 per cent in the total tuberculosis expected during the seven-year period.

Characteristics of the cases: It has been said that BCG vaccination is particularly effective in preventing the more serious forms of tuberculosis, especially miliary disease and meningitis (17). Conversely, it has been maintained that BCG is likely to prevent only the benign forms of tuberculosis (18). In order to examine these questions, and also to see whether the effectiveness of vaccination might have been obscured by the inclusion of doubtful disease, the cases of tuberculosis have been classified according to maximal severity or extent, as shown in tables 12 and 13. In the classification, miliary disease (aside from that occurring only as a terminal event) and meningitis have been assigned precedence over any other form of coexisting tuberculosis. With these exceptions, reinfection-type pulmonary disease has been given precedence over nonpulmonary disease, and cases with other combinations have been assigned to the category which appeared to be the most serious.

In the Puerto Rican study population, tuberculosis mortality was low—5.2 per 100,000 per year. Among the 1,107 cases shown in table 12, only 63 deaths from tuberculosis were reported, a higher ratio of cases to deaths than is usually found in reports to official agencies (19). Fifty of the deaths occurred among reactors. Among controls, 5 deaths were observed, compared with 2 among vaccinees. This

TABLE 13

CLASSIFICATION OF CASES, BY TUBERCULIN AND VACCINATION STATUS, MUSCOGEE-RUSSELL TRIAL

Tuberculosis Classification	Tuberculin and Vaccination Status			
	Total	Reactors	Controls	Vaccinees
Total	206	161	28	17
Pulmonary	115	93	15	7
Far advanced	40	34	3	3
Moderately advanced	38	27	8	3
Minimal	34	30	3	1
Primary	1		1	
Stage unknown	2	2		
Nonpulmonary	17	15	0	2
Miliary, meningeal	8	7		1
Pleurisy with effusion*	2	2		
Lymph node	3	2		1
Bone and joint	1	1		
Renal	2	2		
Pericardial	1	1		
Suspected	74	53	13	8

* Bacteriologically confirmed.

is the most favorable comparison for the effectiveness of vaccination, but there were too few deaths to make the difference statistically significant.

In general, reactors had more extensive disease than nonreactors, largely owing to the higher proportion of cases among nonreactors classified as primary tuberculosis. The type of disease among controls did not differ significantly from that among vaccinees. Cases of miliary and meningeal tuberculosis appeared among all subgroups: 15 among reactors, 2 among controls, and one among vaccinees.

In the Muscogee-Russell trial, a total of 206 cases of tuberculosis was observed among members of the study population, as shown in table 13. Among these cases, only 10 deaths have occurred in seven years. Such a very low tuberculosis mortality rate—2.2 per 100,000 per year—may be an indication of the efficacy of case finding and modern antituberculous therapy.

Aside from the fatal cases, all of which were among reactors, there were no statistically significant differences in the distribution of the kinds of cases among reactors, controls, and vaccinees. Of the 132 classified as definite tuberculosis, 115 were pulmonary and 17 nonpulmonary. Unlike the situation in Puerto Rico, a sizeable proportion of the cases in the Muscogee-Russell trial were classified as suspects, reflecting the unusual interest of the research facility in that area in keeping close observation on all possible cases of tuberculosis. Ninety-four, or

46 per cent, of the 206 cases were confirmed by bacteriologic or necropsy findings: 77 of these were among reactors, 9 among controls, and 8 among vaccinees.[3] No cases of miliary disease or meningitis were reported among controls, whereas one case of miliary tuberculosis occurred in a vaccinee, and 6 cases of miliary disease and one of meningitis among the reactors.

Detailed analysis of these findings shows that the estimated effectiveness of BCG is not materially improved if the marginal cases (primary and suspected tuberculosis) are disregarded. Therefore, it appears that the relatively low effectiveness of vaccination in these trials has not resulted from the inclusion of cases of doubtful disease, which should have been concentrated among the marginal cases. Furthermore, the effectiveness in preventing the most serious forms—fatal, miliary, meningeal, and far advanced tuberculosis—is about the same as for all forms, suggesting that the effectiveness of BCG vaccination is about the same against serious or benign types of disease. The importance of the reactors in the total tuberculosis problem is again emphasized, because they provided the bulk of cases in all categories of serious disease.

Comparison with the British Trial

Among field trials of the usefulness of vaccination against tuberculosis, the one reported by the British Medical Research Council (16) most closely resembles the present trials in concept and execution. In the British trial 56,000 adolescent school-leavers were examined by means of chest roentgenograms and tuberculin tests of 3 and 100 TU. Nonreactors to both doses of tuberculin, who were considered to have normal roentgenograms and no history of contact with tuberculosis, were randomly divided into vaccinee and control groups. Tuberculin reactors were also included in the study population. Although many of the subjects were examined periodically during the follow-up study, somewhat more than half of the cases of tuberculosis were reported by services similar to those used in the Public Health Service trials. Because of the general similarities of the trials, it might have been anticipated that the major results would have agreed and that any significant differences could be accounted for by obvious differences in the study populations or environmental factors.

All three trials agree in showing that tuberculin reactors are subject to high tuberculosis case rates. Moreover, when the cases found by the initial roentgenographic screening are included, agreement between the trials becomes striking in showing that reactors contribute a substantial proportion of the total number of cases appearing in the study populations and that case rates are much higher among reactors than among nonreactors.

With respect to the estimate of the potential usefulness of vaccination, the most recent report of the British trial (21), which covers approximately four years of follow-up observation, indicates that vaccination could have decreased the total number of cases in their study population by more than 40 per cent. In

[3] These cases are comparable with those reported for the Muscogee-Russell trial in a previous paper (20).

the Public Health Service trials, a similar estimate of the usefulness of vaccination showed that less than 10 per cent of the total number of cases could have been prevented even after six to seven years of observation. Only a small part of this difference between the trials can be attributed to the varying proportions of reactors and nonreactors in the study populations or to the differences in case rates in reactors relative to rates in nonreactors. The major source of disagreement appears to lie in the effectiveness of vaccination, obtained by comparing the case rates in control and vaccinee groups. In the British trial, vaccination was reported to be more than 80 per cent effective as compared with a little more than 30 per cent in the Puerto Rican and Muscogee-Russell trials. Reconciliation of this difference does not seem possible, although a number of factors may be considered, such as differences in the vaccines, defects in the trials, and differences in the characteristics of the study populations.

Four vaccines were used in the three trials. In the British study, similar reductions in tuberculosis were obtained with Danish BCG and vole bacillus vaccine. In the present trials, two BCG vaccines produced essentially the same but a much lower reduction in tuberculosis. Had differences in the vaccines been important, some indication of this would have been expected within each set of trials as well as between them. Nor does the ability of the vaccines to produce tuberculin sensitivity appear to shed much light on the discrepant results. Post-vaccinal tuberculin sensitivity was essentially the same in the British and Puerto Rican trials. The percentage of tuberculin conversions attributable to vaccination was much lower in the Muscogee-Russell trial, but no lower than that reported in a trial of vaccination among American Indians in which vaccination was apparently as effective as in the British trial (22). Consequently, it is difficult either to accept or reject differences in the immunizing potency of the vaccines as a cause of significant differences in the observed effectiveness of vaccination.

In the design and execution of these three trials, as in other attempts to measure the effectiveness of vaccination, there are undoubtedly serious defects. To a large extent these defects can be traced to the lack of an adequate placebo for the controls and the consequent impossibility of applying the "double-blind" technique in the conduct of the study. From the preliminary reports of the British trial, it would seem that the subjects of the study must have been frequently reminded of their tuberculin and vaccination status during the course of the periodic follow-up examinations; and that information on the tuberculin and vaccination status of the individual participants was available to the team physicians who made the follow-up examinations and decided which cases would be reported to the Central Office as possible tuberculosis. Thus, even though all cases were assessed in the Central Office by an independent reviewer who did not know the tuberculin and vaccination status of the cases at entry to the trial, it is difficult to see how assurance can be given that bias was avoided in the selection of cases submitted to the reviewer. On the other hand, as discussed in the section on follow-up procedures, entirely satisfactory assurance cannot be given that the Public Health Service trials were free of bias. Nevertheless, it

seems unlikely that bias, mistakes in diagnosis, or other errors in any of the trials could account for even a major share of the difference in the estimated effectiveness of vaccination.

Another difference between the British and the Public Health Service trials is that in both the Puerto Rican and Muscogee-Russell populations low-grade tuberculin sensitivity is highly prevalent, whereas in the British study population such sensitivity is relatively less common. Moreover, in the Public Health Service trials, persons with low-grade sensitivity were included among vaccinees and controls, whereas they were excluded from both of these categories by the British. In a study of tuberculosis in student nurses (23), as well as in the report of the British trial, low-grade sensitivity appears to be associated with resistance to tuberculosis. If, as has been postulated (24), persons with low-grade sensitivity have already had a "natural vaccination," then further vaccination with BCG might not materially increase their resistance. In situations such as pertain in the Public Health Service trials, in which a high proportion of the vaccinees are known to have pre-existing low-grade sensitivity, the observed effectiveness of vaccination might be diminished. Although it is tempting to speculate that differences in low-grade sensitivity among the study populations might account for both a difference in the incidence of tuberculosis among nonreactors and the different estimates of the effectiveness of vaccination, the evidence on this hypothesis is insufficient to consider the matter further at the present time.

Still other differences in the study populations might have influenced the effectiveness of vaccination, and, as illustrated by this brief comparison, much remains to be learned about fundamental problems in tuberculosis. Moreover, the fact that the British and Public Health Service trials, so similar in design and execution, resulted in inexplicable differences in the estimated effectiveness of vaccination emphasizes how injudicious it may be to assume that results obtained in one situation are applicable elsewhere.

Discussion

The most impressive finding of these trials is that the risk of developing tuberculosis is so much greater for reactors than for nonreactors. Although this is consistent with some earlier studies (25–27), it is contrary to the results of others (28–30), and quite the opposite of what was anticipated when the trials were undertaken. At that time, it was widely believed that, if tuberculous disease were to occur, it would generally do so within the first few years after infection; that healthy tuberculin reactors, having passed safely through that period, had acquired resistance to the disease; and that most of the new cases were therefore destined to arise in those not yet infected (31, 32). Indeed, one of the principal arguments for the use of BCG was predicated on that concept of the pathogenesis of tuberculosis (33).

The resistance attributed to reactors, although never regarded as absolute, no longer seems impressive. Results of the present trials and other recent large-scale studies (16, 23, 34) show that the tuberculosis case rates among reactors are far from negligible. One interpretation of this finding might be that case rates

among reactors have increased in recent decades, but this seems highly unlikely. A more credible interpretation is that the incidence of disease among reactors has always been substantial and that it only seemed low and of little consequence in situations in which a large number of cases were arising as a result of new infections. A case rate of 200 per 100,000 among reactors, for example, would seem to indicate considerable resistance if the rate among nonreactors in the same population were 900. On the other hand, a rate of 200 among reactors compared with 40 among nonreactors would not in itself be taken as evidence that reactors had much resistance.

At the present time, there is good reason to believe that case rates among nonreactors in this country are low because the opportunities for new infection have become relatively infrequent and possibly also because of a concomitant decrease in the risk of disease among convertors. Evidence for this conclusion was adduced from the annual emergence of new cases in the present trials. In addition, independent evidence of low and falling infection rates is to be found in reports of numerous tuberculin-testing surveys carried out within the past few years (35–39). This is well illustrated by the results of tuberculin testing of nineteen-year-old white Navy recruits. In 1950, approximately 9 per cent of them were found to be reactors (36), whereas preliminary results of testing in 1958 indicate that approximately 6 per cent are reactors (39). The implication of this decrease is that the present infection rate in this country can hardly be more than 1 per 1,000 per year.

The most reasonable interpretation, then, for the divergence of present findings from what was generally expected is that, although tuberculosis case rates for reactors have not changed very much over the years, the case rates for nonreactors during the same period have decreased markedly. Furthermore, it appears that the low case rates among nonreactors can be attributable primarily to the present low rate of acquiring new infections.

Since the infection rate affects the amount of tuberculosis available for prevention by vaccination, it may be generalized that, other things being equal, the usefulness of vaccination will vary directly with the infection rate. In the present trials, the infection rates that prevailed during the period of observation plainly influenced the estimates that vaccination could have reduced the total amount of tuberculosis in the study populations by less than 10 per cent. Had the infection rates been lower, or had they declined more sharply, vaccination would have been even less useful. It should be apparent, also, that low infection rates in any population group are, to a considerable extent, a direct result of the successful application of other methods of tuberculosis control—case finding, isolation, and treatment of active cases of the disease.

The usefulness of vaccination is also dependent on the proportion of nonreactors in a population. If this proportion is large, it is possible, even with low infection rates among nonreactors and low case rates among those who become infected, that a major part of the tuberculosis problem will be susceptible to attack by vaccination (34). But it is equally clear that, whenever the infection rates are low, the absolute magnitude of the tuberculosis problem among non-

reactors will also be low, and consideration must be given to the disadvantages of vaccination. With falling infection rates, a point will be reached in any population when the disadvantages of vaccination outweigh the advantages. Recognition of the cogency of this argument is, of course, the basis for recent suggestions that the time has come to curtail vaccination in the Scandinavian and other countries where infection rates, as in this country, have become very low (40).

Among groups subjected to extremely low infection rates, it is conceivable that the clinical complications of vaccination, uncommon though they may be (41), might counterbalance the gains of preventing a few cases of tuberculosis. Long before this point is reached, however, the really significant penalty of vaccination—*its interference with the interpretation of the tuberculin reaction*—becomes of crucial importance. Some BCG vaccines produce tuberculin sensitivity which is indistinguishable from that resulting from natural infections (42); but even when post-vaccinal sensitivity is weak, it is rarely possible in an individual case to distinguish between tuberculin sensitivity resulting from natural infection and that from vaccination, or, indeed, to detect the presence of superinfection in a vaccinated person. The tuberculin sensitivity conferred by vaccination thus makes it impossible to identify the occasional persons who become infected. For vaccinated persons, both those who do and those who do not become infected, the differential diagnosis of pulmonary disease is made more difficult, perhaps for the rest of their lives.

An equally serious penalty of BCG vaccination is interference with the use of the tuberculin test for epidemiologic and case-finding purposes. At a time when it is becoming increasingly evident that natural tuberculin reactors have a much greater risk of developing tuberculosis disease than nonreactors, and when the number of reactors in many areas is becoming small enough so that they may be kept under surveillance (and perhaps given prophylactic treatment), any procedure which destroys the method of identifying such persons must be regarded as a backward step in tuberculosis control.

From a consideration of these factors, it is apparent that extensive vaccination is not indicated in this country, an opinion shared by most tuberculosis workers (43, 44). Faced with the fact that most cases of tuberculosis today are arising in tuberculin reactors in the older age groups, some workers have suggested that vaccination should be done at birth or during infancy (45). This argument might have had some merit in the early part of the century when infection rates were high. By 1950 it was already much too late. By that time, despite the high prevalence of tuberculin reactors in the older age groups, the infection rate had already fallen to the point where few of the current generation of infants and children risk a tuberculous infection during their lifetime. For all but those few, vaccination could only be a disadvantage.

Aside from its use in field trials, BCG vaccination in the United States has been limited to special groups—nurses, medical students, laboratory workers, and others presumed to be at considerable risk of developing tuberculosis because of their environment. Recently, and particularly since the publication of the preliminary report of the British trial (16), there has been some concern because

BCG has not been used extensively even among these special groups. However, the risk among such groups is by no means uniform (46, 47), and in many instances it is already low (23, 48–50). The latest recommendations on the use of BCG in the United States take cognizance of the rapidly changing conditions and mention special groups only as examples of situations in which the risk of infection *may* be high (44).

Before embarking on a vaccination program for any group, it would seem essential to ascertain both the present and probable future risk of infection. Not only does this factor have the most immediate influence on the usefulness of BCG, but it also has a pronounced bearing on the degree of interference with the interpretation of the tuberculin reaction. Obviously, if nearly all individuals in a group will become reactors in a few years as a result of natural infection, the creation of artificial sensitivity is not a serious handicap. And, if it appears that the tuberculin test cannot be put to use in controlling tuberculosis, or if the sources of infection, even if located, cannot be removed, then the protective effect of vaccination—be it high or low—is clearly a gain. But it should also be recognized that vaccination, even under these circumstances, is a tacit admission of defeat, since it implies a situation so out of hand that present-day tuberculosis control methods cannot be satisfactorily applied. In this country today, where the biologic balance has already been tipped so strongly against the survival of the tubercle bacillus, nothing should be allowed to interfere with modern methods of case finding, isolation, and treatment. If these procedures are diligently applied to the tuberculin reactors of today, then their passing from the scene should mark the virtual eradication of tuberculosis.

Summary

A progress report has been made after a follow-up period of six to seven years of two controlled trials of BCG vaccination, initiated and directed by the Public Health Service. One trial, on children aged one to eighteen years, is in Puerto Rico, an area where tuberculosis has been a serious problem for many years; the other, on persons more than five years of age, is in the community formed by Muscogee County, Georgia, and Russell County, Alabama, where the tuberculosis problem is similar to that found in many other communities in this country. More than a quarter of a million persons were placed under study: 112,000 tuberculin reactors, and 144,000 nonreactors who were allocated by a randomization scheme to vaccinated and control (unvaccinated) groups. For the identification of new cases of tuberculosis appearing in the study populations, the established medical, public health, and vital statistics reporting systems were deliberately chosen as being sufficient for the purposes of the study, and also as being more likely to yield unbiased information than systematic periodic examinations. Certain policies and techniques were adopted as safeguards against the introduction of bias in the diagnosis and reporting of cases.

The most striking finding of both trials was that the risk of developing tuberculosis was much greater for persons who were tuberculin reactors on entry than for those who were nonreactors. Of the total number of cases that appeared

during the follow-up period, 75 per cent were among reactors; consequently, only the 25 per cent of the cases that would have appeared among the initial nonreactors could have been prevented if vaccination had been completely effective.

Tuberculosis case rates among nonreactors were low. In Puerto Rico the rate was 43 per 100,000 per year among controls and 30 among vaccinees. The difference, representing 31 per cent fewer cases among the vaccinees than among the controls, is statistically significant. In the Muscogee-Russell trial, the corresponding rates were 22 among controls and 14 among vaccinees, but the difference (36 per cent) is not statistically significant. If all nonreactors had been vaccinated, the total number of cases in the study populations would have been reduced by 8 to 9 per cent—an estimate obtained by applying a reduction of 31 to 36 per cent to the 25 per cent of the cases which would have appeared in those who could have been vaccinated.

The low case rates among nonreactors can be directly attributed to the present low risk of acquiring new infections. Evidence of low and falling infection rates in this country is found both in the present trials and in other recent studies. Because BCG cannot help those who are already infected, nor those who will not become infected, and may be helpful only to a portion of the decreasing few who will become infected in the future, it is apparent that vaccination cannot be very useful in controlling tuberculosis in this country. Moreover, with the rapid decline in tuberculous infection, the tuberculin test is becoming increasingly more valuable for epidemiologic, case-finding, and diagnostic purposes. These uses of the tuberculin test are destroyed by vaccination, which makes it virtually impossible to identify the naturally infected persons. And, as those who are already infected are now the group at greatest risk, it is upon them that tuberculosis control activities should be focused if the disease is to be eradicated. The position is taken that in most situations in this country today the advantages of vaccination are outweighed by the disadvantages.

Sumario

Pruebas Colectivas de la Vacunación con BCG

Este informe preliminar ha sido preparado a continuación de un período de seis a siete años de observación de dos ensayos fiscalizados de vacunación con BCG, iniciados y dirigidos por el Servicio de Sanidad Pública de los E. U. A. Un ensayo, en niños de uno a dieciocho años, es en Puerto Rico, donde la tuberculosis ha constituído un problema de gravedad por muchos años; el otro, en personas de más de cinco años de edad, es en la colectividad formada por el Condado de Muscogee, Georgia, y el Condado de Russell, Alabama, en la que el problema tuberculoso es semejante al observado en otras muchas colectividades del país. Más de 250,000 personas figuraron en el estudio: 112,000 reactores a la tuberculina y 144,000 que no reaccionaron, asignados todos por medio de un plan de selección al azar a grupos de vacunados y testigos (no vacunados). Para la identificación de los casos nuevos de tuberculosis que aparecían en las poblaciones en vías de estudio, se escogieron deliberadamente los sistemas establecidos de notificación, médicos, sanitarios y demográficos, como suficientes para los fines del estudio, y también como más susceptibles de ofrecer información imparcial que los exámenes periódicos sistemáticos. Como salvaguardias contra la introducción de prejuicios en el diagnóstico y la declaración de los casos, se adoptaron ciertos principios y técnicas.

El hallazgo más notable de ambos ensayos fué que el riesgo de contraer la tuberculosis era mucho mayor para los que reaccionaban a la tuberculina al ingreso que para los que no reaccionaban. Del número total de casos que aparecieron durante el período de observación, 75 por ciento fueron entre los reactores; por consiguiente, sólo hubiera podido impedirse el 25 por ciento de los casos que hubiese aparecido entre los que no reaccionaron al principio si la vacunación hubiese sido absolutamente eficaz.

Las tasas de casos de tuberculosis entre los que no reaccionaron fueron bajas. En Puerto Rico, la tasa fué de 43 por 100,000 al año entre los testigos y de 30 entre los vacunados. La diferencia, que representa 31 por ciento menos casos entre los vacunados que en los testigos, es importante estadísticamente. En el ensayo de Muscogee-Russell, las tasas correspondientes fueron de 22 en los testigos y 14 en los vacunados, pero la diferencia (36 por ciento) no es importante estadísticamente. Si se hubiere vacunado a todos los que eran reactores, el número total de casos en las poblaciones estudiadas se habría rebajado en 8 a 9 por ciento —cómputo este obtenido aplicando una rebaja de 31 a 36 por ciento al 25 por ciento de los casos que habrían aparecido en los que no pudieron vacunarse.

Los bajos coeficientes de morbidad entre los que no eran reactores pueden imputarse directamente al bajo riesgo actual de adquirir infecciones nuevas. Pruebas de las bajas y descendentes tasas de infección en este país las ofrecen tanto los ensayos actuales como otros recientes estudios. Como el BCG no puede ayudar a los ya infectados, ni tampoco a los que no se infectarán, y puede resultar útil únicamente a una porción a los pocos y cada vez menos que se infectarán en el futuro, es manifiesto que esa vacunación no puede ser muy útil en el domibio de la tuberculosis en los E. U. A. Además, con el rápido descenso de la infección tuberculosa, la prueba de la tuberculina se va volviendo cada vez más valiosa para fines epidemiológicos, de descubrimiento de casos y de diagnóstico. Estas aplicaciones de la prueba de la tuberculina son aniquiladas por la vacunación, que virtualmente imposibilita la identificación de las personas infectadas naturalmente. Y, como los ya infectados constituyen hoy día el grupo más en peligro, es en ellos que hay que concentrar las obras de lucha antituberculosa si va a erradicarse la enfermedad. Se toma la actitud de que, actualmente en la mayor parte de las situaciones en este país, las ventajas de la vacunación con BCG quedan contrapesadas por las desventajas.

Resume

Essais de vaccination collective par le B.C.G.

Il s'agit d'un rapport sur l'evolution de deux essais contrôlés de vaccination collective par le B.C.G. L'étude a été instaurée et dirigeé par les Service Publics. Un essai sur des enfants de un à dix-huit ans est en cours a Porto-Rico, region où la tuberculose constitue un grave problème depuis de nombreuses années; l'autre concerne des sujets âgés de plus de cinq ans dans une collectivité constituée par le Muscogee County, Georgia, et Russell County, Alabama: Dans ces collectivités le problème de la tuberculose est semblable à celui qui se pose pour de nombreuses municipalités des Etats-Unis. Plus de 250.000 personnes ont été mises en observation: 112.000 sujets avaient une réaction positive à la tuberculine et 144.000 une réaction négative; ces cas ont été répartis au hasard dans les groupes vaccinés et les groupes témoins (non vaccinés). Les systèmes classiques d'ordre médical, hygiène publique et statistique (Etat-Civil) pour l'identification des nouveaux cas de tuberculose ont été délibérément adoptés; ils ont été considérés valables pour l'objectif de l'étude et aussi pour fournir des informations oú le risque d'erreurs systématiques est moindre que celui que présenté par les examens périodiques de dépistage systématique. Certaines dispositions et techniques ont été appliqéeés pour éviter l'introduction d'erreurs systématiques dans le diagnostic et l'évaluation des cas.

La découverte la plus importante de cette étude a été que le risque du développement de la tuberculose était beaucoup plus grand chez les sujets à cuti positive, au moment de leur incorporation dans l'étude, que pour les sujets "négatifs." Parmi la totalité des cas décelés au cours de la période d'observation, 75% était des sujets cuti-positifs; en consé-

quence, 25% seulement des cas qui se seraient développés parmi les sujets initialement "négatifs" auraient pu être prévenus si la vaccination avait été complètement efficace.

Les taux des cas de tuberculose parmi les sujets "négatifs" étaient faibles. A Porto-Rico, le taux était de 43 pour 100.000 par an dans le groupe témoin non vacciné et de 30 pour 100.000 dans le groupe vacciné. La différence, représentant 31% de cas en moins parmi les sujets vaccinés que chez les sujets témoins est significative du point du vue statistique. Dans les essais de l'étude Muscagee-Russell, les taux correspondants étaient 22 chez les sujets témoins et 14 chez les sujets vaccinés, mais le différence (36%) n'est pas significative. Si tous les sujets négatifs avaient été vaccinés, le nombre total des cas dans la population étudiée eut été réduit de 8 à 9%—estimation obtenue en appliquant une réduction de 31 à 36% aux 25% des cas susceptibles d'être apparus parmi ceux qui auraient été vaccinés.

Les taux bas parmi les sujets "négatifs" peuvent être directement attribués au risque actuellement réduit d'acquisition de la tuberculose. La démonstration de taux bas et décroissants de la tuberculose aux Etats-Unis est évidente à la fois dans les essais précités et autres études récentes. Etant donné que le B.C.G. ne peut aider ni ceux qui sont déjà infectés ni ceux qui peuvent être contaminés et ne peut être utile qu'à la fraction du petit nombre constamment décroissant de ceux qui seront contaminés dans l'avenir, il est évident qu'aux Etats-Unis la vaccination ne saurait être très utile pour lutter contre la tuberculose. De plus, avec le déclin rapide de l'infection tuberculeuse, la valeur du tuberculine-test devient de plus en plus grande à l'égard de l'épidémiologie, du dépistage et du diagnostic. Ces applications du tuberculine-test sont annulées par la vaccination, qui rend pratiquement impossible l'identification ces cas d'infection naturelle. De plus, comme ceux qui sont déjà infectés constituent actuellement le groupe du plus grand risque, c'est chez ces sujets que les mesures de la lutte contre la tuberculose doivent être centrées pour réaliser l'éradication de cette maladie. La conclusion est que dans la plupart des conditions existant aux Etats-Unis aujourd'hui, les inconvénients de la vaccination l'emporte sur ses avantages.

Acknowledgments

The initiation and continued conduct of these studies were and still are dependent on the cooperation and participation of many persons. It must suffice to express the gratitude of the writers publicly to only a few key persons, although they are also greatly indebted to their associates and subordinates.

In Puerto Rico, special recognition should be given to Dr. Juan A. Pons, Secretary of Health, who, at a time when enthusiasm for mass vaccination campaigns was running high, had the foresight and courage to support a controlled trial. Other staunch supporters of the program were Dr. Guillermo Arbona, who recently succeeded Dr. Pons as Secretary of Health, and Mr. Mariano Villaronga, Secretary of Education. Members of the Puerto Rico Medical Association also deserve much credit for their efforts to dispel the fears created by adverse publicity. The actual field operations were ably supervised by the late Dr. Eduardo Garrido Morales, and the unusually thorough follow-up record system was developed and managed by Mrs. Verna T. Livesay.

In the Muscogee-Russell trial, Dr. James A. Thrash, Commissioner of Health for Muscogee County, has been in large measure responsible for the success of this and other research programs in the County. Dr. H. C. Schenck, Director of the Division of Tuberculosis Control, Georgia Department of Public Health, strongly supported the work during this period, as did Dr. W. B. Mims, Jr., Health Officer of Russell County. The vital spark needed to coordinate the activities of the various agencies participating in the testing and vaccinating phase of the trial was supplied by the Chattahoochee Health Foundation, a group of dedicated volunteer workers headed by Mr. J. Q. Davidson.

The technical phases of both trials were planned and directed by the staff of the Tuberculosis Program, U. S. Public Health Service. Dr. Harold S. Barrett, now Deputy Com-

missioner of Health of Connecticut, deserves special mention for his work in coordinating the field activities of both trials.

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ANIMAL TUBERCULOSIS[1, 2]

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(Received for publication October 28, 1957)

INTRODUCTION

Tuberculosis is believed by some authorities to have affected animals that inhabited the earth before the advent of man. Animal bones of the paleolithic and neolithic periods show evidence of the disease, and recorded history from very ancient times contains unmistakable references to its effects on man.

Proscriptions against eating the flesh of animals with disease now believed to have been tuberculosis appeared in the Jewish Talmud in the second and fifth centuries A.D. Laws concerning the use of diseased animals were enacted by German cities as early as the fourteenth century. In 1732, the Germans, believing that bovine tuberculosis and syphilis were related or identical, ordered the destruction of all tuberculous cattle and imposed penalties for the sale of meat from infected animals. These regulations were repealed some fifty years later after Graumann demonstrated that the two diseases were different and concluded from that that the meat of tuberculous cows was safe for human consumption (1). Almost a hundred years were to pass before the wisdom of these discarded public health practices and other preventive veterinary medical measures was again recognized. A number of French, German, and English veterinarians and physicians recognized the infectious nature of tuberculosis in animals and man in the early nineteenth century, but this concept was debated for many decades. As early as 1850, German veterinarians were advising dairy farmers to dispose of their tuberculous animals (2). By 1870—some twenty years before pasteurization of milk was accepted as necessary to protect the human consumer—the infectiousness of tuberculous cows' milk for swine and calves was suspected, and Bavarian farmers were urged to cook milk before feeding it to young animals. Koch's discovery in 1882, of course, made possible the differentiation of the three types of tuberculosis organisms: human, bovine, and avian. For some time at the turn of the present century, controversy raged about whether the bovine tubercle bacillus was infectious for man before its seriousness as a human health hazard was generally accepted. Now it is known, of course, that bovine tuberculosis can be transmitted to man both by the ingestion of milk from tuberculous cows and by inhalation of bacilli in droplets expelled by animals' coughing.

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[2] Presented before the Medical Section at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 8, 1957.

Tuberculosis in Cattle

The British Royal Commission on Tuberculosis pointed out the danger of cow's milk as a cause of tuberculosis and fatal tuberculosis in man and urged that stringent measures be taken to prevent the sale and consumption of such milk. The persistent and meticulous work and the conservative interpretation of the results, by Griffith, were probably more responsible for the final indictment of *Mycobacterium tuberculosis bovis* as a menace to human health than were the efforts of any other individual. The conclusions of Griffith and Munro (3), in their extensive study on human pulmonary tuberculosis of bovine origin, further emphasized the danger of milk of tuberculous animals and revealed that pulmonary tuberculosis in man often resulted from the ingestion of milk containing living bovine bacilli. They predicted that this form of disease in man would disappear if infected milk from tuberculous cows was not used for human consumption.

The control of bovine tuberculosis in animals was begun in the United States shortly after tuberculin was discovered by Koch in 1890 and demonstrated by Bang to be an effective agent in the identification of tuberculous animals. Many health departments in the Midwest began to test milk cows for tuberculosis shortly afterwards. This testing eventually led to a national program in 1917 under the U. S. Department of Agriculture. About the same time that testing of milk cows was started in the Midwest, pasteurization of milk was introduced in New York City. Within a few years pasteurization was made compulsory in many cities and towns. Chicago and Minneapolis were among the first cities to adopt compulsory pasteurization during the first decade of the twentieth century. There is no better example of its effectiveness in eliminating tubercle bacilli from a large city milk supply than the reports of Price (4). In 1916 Toronto made pasteurization compulsory. Between that time and 1939 there was not a proved case of bovine tuberculosis in a generation of children using city milk.

The more susceptible breeds usually succumb to tuberculosis within weeks. The British Royal Commission on Tuberculosis (5, 6) reported in 1917 that 94 per cent of Jersey calves given subcutaneous injections of bovine tubercle bacilli were dead within ninety days.

Most authorities agree that cattle are much more susceptible to infection by the respiratory than by the alimentary route. The distribution of lesions in cattle, as shown by meat inspection reports, leaves no doubt that thoracic disease is much more common than disease in the abdominal organs. Theobald Smith and later investigators who looked into this problem agree that the transfer of infection occurs in the stable or in other close confinement, when diseased cattle are coughing and expelling infectious droplets.

Tubercle bacilli are found in cattle feces which are the source of infection for other animals, especially swine because of their coprophagic habits. Dried feces clinging to the udder, tail, and flanks of milk cows are a major source of contamination for milk, even more so than infection within the udder, which is

infrequent. Dried fecal dust may also be a source of milk contamination in the barn or barnyard.

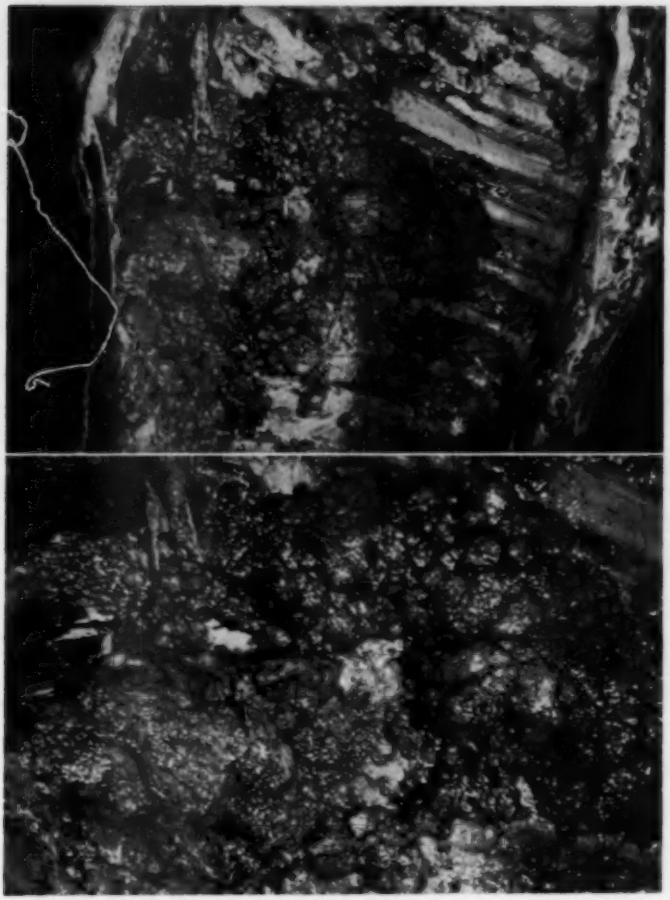
Today most investigators of animal tuberculosis believe that a primary complex occurs in all animals at infection and that, whenever tubercle bacilli invade the animal host, there is some evidence of their activity. When the activity is arrested the primary lesions may disappear, but observations of tuberculous disease in calves reveal that the primary focus usually remains latent. Such lesions are quite often active, a fact supported by epizootiologic data, which indicate that, if any infected animals are left in a herd, others are likely to become infected. Primary lesions in cattle may remain quiescent for long periods, after which progressive disease develops. (It is also possible that, when primary lesions heal, endogenous reinfection occurs.) From the primary lesion, bacilli spread through the lymphatic vessels or through the bronchi to other parts of the lung.

Although pulmonary tuberculosis in cattle may cause an acute caseating pneumonia, it usually takes a more chronic form. The individual lesions of the chronic form often include a limited area of bronchopneumonia. True primary cavitation rarely occurs in cattle, although it is common in cats and dogs. The pleural lesions are quite characteristic. They first appear as soft, pink, velvety growths and later they develop into grapelike clusters (figures 1, 2).

Medlar in his excellent report on tuberculosis in cattle (7) points out that in animals more than six months old the disease is primary in the lung and is in all probability contracted by inhalation of the bacilli. In Medlar's study of 520 pairs of tuberculous lungs, it was quite evident from the haphazard distribution of lesions in the lung parenchyma and their location that the infection was air borne. Single tuberculous lesions were found in all regions of both lungs. Multiple lesions showed a similar haphazard distribution. In most instances the lesions were in the superficial portion of the lung parenchyma; few were in the deeper portion. Medlar described the various macroscopic lesions as follows: very slight caseation (early); soft caseous; firm caseous; fibrocaseous; calcified; and cavitating. Cavitation was frequently demonstrated and was described by Medlar as a "very common occurrence." To what extent this cavitation is primary is impossible to determine but, inasmuch as most of the 2,005 lesions that were described were in the superficial parenchyma, it would follow that the cavitation was the result of the extension of these lesions.

Endobronchial lesions were observed frequently in advanced pulmonary disease in cattle in connection with cavitation. Tuberculosis of the abdominal viscera was a common occurrence in the far advanced cases in this series with cavitation. From this it is apparent that the intestinal tuberculosis was secondary to the ingestion of tuberculous pus from a cavitating pulmonary lesion.

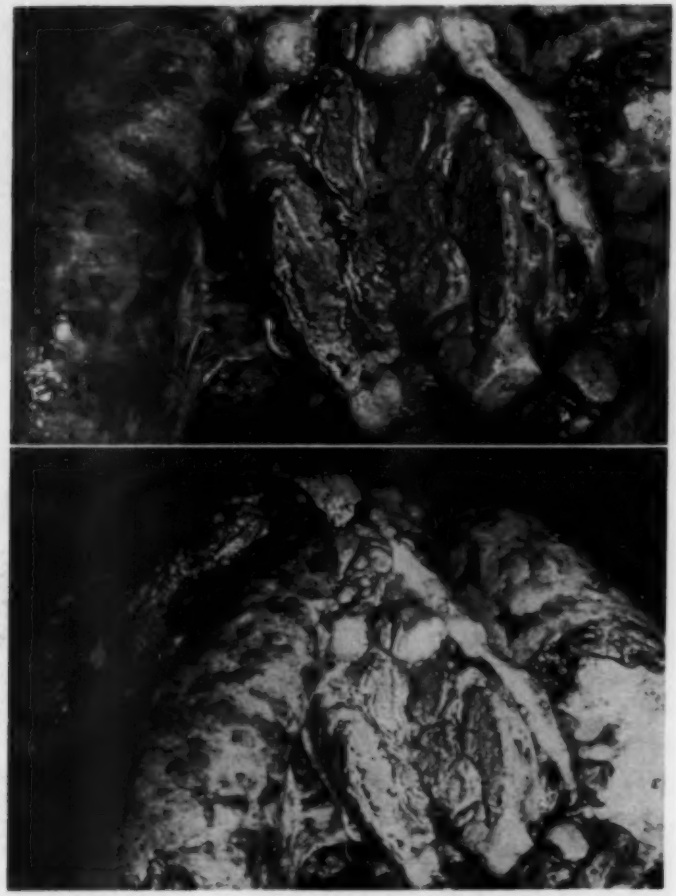
Lesions produced in calves by experimental subcutaneous infection with bovine tubercle bacilli are extensive at the site of inoculation. An enormous necrotic tumor develops and infiltrates the covering skin and subjacent muscles. Such local tumors remain hard. The approximal lymph nodes become enlarged and quite firm. The lungs are extensively diseased, with small, opaque, grayish tubercles scattered very thickly. Around these tubercles



FIGS. 1 and 2. Photomicrographs showing characteristic pleural lesions which appear as soft, pink, velvety groups and later develop into grapelike clusters.

are frequently congested, edematous areas that are confluent, so that the lung tissue contains little air. Tubercles are also numerous in the liver, spleen, and kidneys. Every lymph node may be affected, and the mediastinal lymph nodes in the chest enormously enlarged (figures 3, 4). In more chronic cases, "pearly disease" growths are common on the margins of the lungs and on the serous lining of the thoracic walls, as in naturally occurring disease.

When calves are challenged subcutaneously with *human* tubercle bacilli, the disease does not usually spread beyond the site of inoculation and the nearest lymph nodes. A recent report suggests that palmitic acid may be the active component in bovine tissue underlying the resistance of cattle to human tubercle bacilli (8). In rare instances the disease becomes generalized and persists for



FIGS. 3 and 4. Photomicrographs showing caseated mediastinal lymph nodes.

years. Traum (9) encountered a case in 1916 in which a human tubercle bacillus was isolated from the milk of a cow three years after the animal had been inoculated as a calf for the purpose of producing artificial immunity (10). British investigators have also pointed out the dangers of immunizing milk cows with living tubercle bacilli (11). When human tubercle bacilli are injected directly into the blood stream, they will produce a pneumonia that can be rapidly fatal.

Reports on the isolation of human tubercle bacilli from naturally infected cattle indicate that the associated lesions are usually slight. The infection is important chiefly in sensitizing the animal to tuberculin. When animals are sensitized under such conditions, they must be treated as diseased animals and removed for slaughter.

Cattle rarely contract tuberculosis from man, although this occurs in the United States today more often than man contracts tuberculosis from animals. During the last two years, epidemiologic reports of tuberculous infections in cattle contracted from humans have been received from South Dakota, Missouri, and Michigan. One of the cases reported by the Michigan Veterinary Reporting Service was attributed to a dairy owner who was hospitalized with tuberculosis of the scrotum. Fourteen of the 20 cattle in his herd were reactors to the tuberculin test. On slaughter the 14 animals showed no visible lesions, although the tuberculin test reactions had been as large as a fist. Another case reported from Michigan involved a 16-year-old boy found to have moderately advanced pulmonary tuberculosis. He had taken care of a dairy herd which, when tested, produced 2 reactors out of 15 animals. At slaughter the reactors showed no visible lesions. The remainder of the herd was also slaughtered and no evidence of the disease was seen. The instances reported from South Dakota and Missouri were similar to those in Michigan.

Feldman (12) has reviewed infection of cattle with avian tubercle bacilli in his excellent monograph. Usually the tissue changes are slight, but severe generalized disease is reported occasionally, including mastitis and metritis which may give rise to abortion and even to congenital infection. Avian tubercle bacilli are an important cause of nonspecific sensitization to mammalian tuberculin.

Hagan and associates, as well as many other investigators, believe that contact with acid-fast organisms other than tubercle bacilli are able to sensitize cattle to tuberculin (13).

Bovine Tuberculosis in Humans

Human infection with bovine tubercle bacilli is exceedingly rare in the United States today. Only isolated cases are known to have occurred in this country in the past seven years. An example of a recent case confirmed by the Communicable Disease Center, Public Health Service, was one reported in 1954 in a 6-year-old boy who lived on a farm in Michigan. Tuberculin testing of the animals on the farm revealed that all of the cattle were reactors, and the slaughter report indicated that gross lesions had been found in 15 of the 17 infected cows. (Since the presentation of this paper a case has been reported in a packing house employee in Massachusetts.)

Other single cases of human infection have been reported since 1950 from Maine, Connecticut, West Virginia, and Illinois. The only outbreak in recent years was in northern Ohio in 1948; it involved 119 schoolchildren, a number of whom developed lymphadenitis. In no instance was it possible to isolate an etiologic agent from the lymph nodes, but the histopathologic findings were compatible with a diagnosis of tuberculosis. Bovine tuberculosis as a cause of tuberculous lymphadenitis in humans is frequently discussed, but there are few cases in which the relationship is proved by bacteriologic study.

Fortunately in the United States, the bovine type of pulmonary disease is unknown. Griffith and Munro (3) have reported on the spread of bovine tuberculosis from man to cattle in Great Britain where human disease of this type

formerly existed. Likewise Sigurdsson (14) has reported similar instances in Denmark.

While the human cases of bovine tuberculosis that have come to the attention of the present writers in recent years do not represent all that have occurred in this country, the fact that there are so few known instances does give some index of how infrequently the disease occurs. This favorable situation, of course, is a result not only of the common practice of pasteurizing milk, but of the campaign to eradicate tuberculosis in cattle, begun forty years ago by the United States Department of Agriculture in cooperation with the states.

Eradicating Tuberculosis in Cattle

Progress

Although the goal of complete eradication has not yet been reached, progress has been noteworthy. About 378 million tuberculin tests have been made on cattle in the past forty years, and slightly more than 4 million reacting animals have been found and sent to slaughter. In almost every year since the eradication campaign got under way in 1917, the incidence of the disease, as indicated by tuberculin test results, has been reduced below the level of the previous year. In 1918—the peak year—nearly 5 per cent of the animals tested were found to be infected with tuberculosis. In some milk sheds the rate of infection at that time ranged from 25 to 50 per cent. By 1957, the rate of infection for the country as a whole had declined to 0.15 per cent—3 reactors of every 2,000 cattle tested.

A gradual reduction from year to year in the number of cattle carcasses that show lesions of tuberculosis under federal meat inspection (not including reactors sent for slaughter) has coincided with the decrease in the percentage of reacting cattle. It has been calculated that nearly 100,000 carcasses would have been condemned as unfit for human consumption in 1957 if bovine tuberculosis had remained unchecked since 1917. The actual number so condemned in 1957 was 212. On the basis of current prices, the savings on the condemnations resulting from the testing program and on the killing floor equal more than $150 million a year. The total expenditure for the eradication program over the years has been $326 million. Thus, in two years the program saves almost as much as has been spent for it in forty years. However, despite these signs of major progress, bovine tuberculosis still occurs in this country and probably affects some 140 thousand cattle[3] which as yet have not been identified (figure 5).

In 1957, almost 10 million cattle (10 per cent of the total cattle population) were tuberculin tested. Of the 14 thousand reacting animals, fewer than one-half revealed lesions pathognomonic of bovine tuberculosis on slaughter. Many of the lesions found were in the early stages, indicating recent infection. As

[3] One hundred and forty thousand infectious cattle is an estimate based on an extrapolation of 0.15 per cent reactors among 10 million cattle tested in 1957 out of a total population of 100,000,000. It is presumed that the 0.15 per cent reactor rate would apply to the total cattle population.

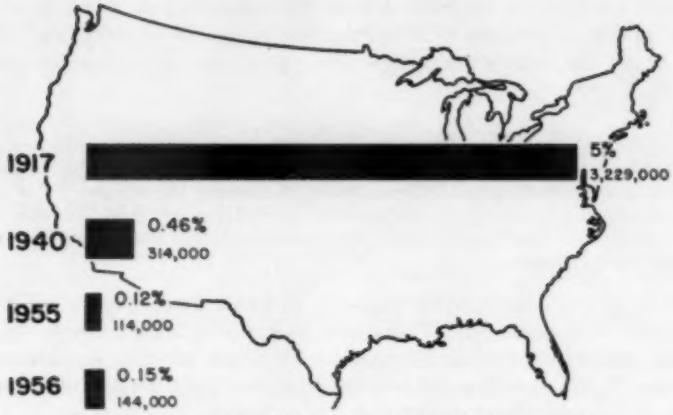


FIG. 5. Bovine tuberculosis: Infected animals (calculated).

the incidence of infection has been reduced, opportunity for exposure lessened, and factors responsible for nonspecific reactions have tended to remain fairly constant, a sharply increasing proportion of reactors slaughtered have shown no visible lesions. Reaction in these animals is due in part to exposure to: (*1*) human tubercle bacilli; (*2*) avian tubercle bacilli; (*3*) *Mycobacterium paratuberculosis;* and (*4*) nonspecific acid-fast organisms. Health and agricultural agencies are intensifying their epidemiologic and epizootiologic investigations to determine the etiology of tuberculin reactions in animals when no-visible-lesions are found.

During the early years of the eradication campaign, emphasis was placed on building up a large number of individually accredited herds. (An accredited herd is one that has been tuberculin negative on two successive annual tests.) As soon as possible, states adopted area testing, usually on a county basis. In 1940, twenty-three years after the program began, the last county in the United States became a modified accredited area, that is, one showing less than 0.5 per cent of tuberculosis. This achievement was only an intermediate goal. It is now necessary to think in terms of complete eradication in all states.

Since the entire nation has reached a modified accredited status, a tendency toward complacency among members of the livestock industry in some areas and among some regulatory groups has been deterring the eradication effort. Research, education, and improved testing procedures have helped to bring the incidence of tuberculosis among cattle to an extremely low level in this country. Further research and education, particularly the kind of education that counters apathy, will be needed to accomplish eradication.

In many states there is a close working relationship between public health officials and state and federal livestock sanitary officials, who keep each other fully informed of any possible relationship of tuberculosis in humans and in animals. This close contact should be extended to all states. A sudden realiza-

tion that a few herds are riddled with tuberculosis alerts the general public to the danger of allowing a little infection to remain uncontrolled. There is little doubt that public health groups are aware of and sympathetic to this problem.

Procedures

Scientifically accurate testing and interpretation of results, strict quarantine of infected herds after reactors are removed, thorough cleaning and disinfection of premises which have been occupied by infected animals, determining the type of infection and the origin of infected animals, and tracing down exposed animals to locate new foci of infection are all necessary measures.

For many years there was a tendency to test a herd, remove the reactors, and pass on to the next herd. Today more attention is being given to the epizootiologic investigation of all infected herds. When infection is scattered as it is now, nothing is an effective substitute for searching out and destroying the disease completely where and when it can be found.

A slight increase in the annual infection rate last year was probably due to tracing to herds of origin animals that showed lesions of tuberculosis on slaughter, along with similar follow-up study of those that reacted to the tuberculin test, and the testing of exposed animals. Such temporary increases in infection rates are to be expected, as the centers of infection are more effectively located through established epidemiologic procedures.

The manner in which tracing can increase the number of known reactors is illustrated by an incident that occurred last July in Ohio. A producer sold his entire herd of 88 beef cattle, expecting them to be sent to immediate slaughter. Instead, they were auctioned, 48 of them going to twelve different slaughterhouses and the remaining 40 going to three farmers and three dealers. Federal inspectors at one plant and municipal inspectors at two plants reported 5 carcasses showing lesions of tuberculosis. Acting on this information, federal and state tuberculosis eradication officials traced the animals, now scattered to four additional farms. In all, the investigators found 23 reactors on seven premises. Tracing and testing were completed in three weeks from the date the animals were sold.

Another example—which demonstrated the value of tracing reactors to locate new centers of infection—concerns a Connecticut dealer's herd. Eight reactors were found when this herd was tested in May, 1955. Five of the reactors had originated in Maine, 2 in Connecticut, and one had previously been tested in the dealer's herds. Investigations of herds from which all reactors had come turned up no infected animals. However, as a result of routine area testing and the tracing of animals that had moved through the herd during the previous six-month period, infection was found in three states: 7 reactors in four Connecticut herds, 7 in three Massachusetts herds, and 6 in two herds in Rhode Island.

Because epizootiologic tracing so frequently pinpoints the source of infection, it turns up a higher percentage of reactors than does testing animals for other reasons. For example, during the calendar year 1956, testing the herds of origin

TABLE 1

TUBERCULIN TESTING OF CATTLE, JANUARY 1–DECEMBER 31, 1956

Reason for Tests	Number Tested	Number of Reactors	Number of Cattle Tested to Find One Reactor
Area testing and herd accreditation	7,413,105	8,336	889
Quarantine herds	365,880	3,666	99
Testing herds after tracing diseased or exposed animals	62,234	1,061	58
Animals tested for sale or to comply with interstate requirements	489,076	119	4,109
Other (miscellaneous)	658,639	616	1,069

of carcasses found to have tuberculous lesions at slaughter turned up one reactor in every 60 cattle tested in such herds; testing quarantined herds turned up one reactor in every 99. Herd accreditation tests, on the other hand, revealed one reactor in every 3,052 cattle tested.

When reports submitted by the Meat Inspection Division of the Department of Agriculture and by state and local meat inspection agencies are used to identify and locate the herds from which tuberculous animals have come, and those herds are tuberculin tested, the spread of infection can be minimized by eliminating other diseased animals before they are sold and moved into other herds. This procedure is both direct and economical. In one state, during the calendar year 1953, the cost of locating a reactor by tracing and testing was calculated to be $32, while the cost of locating a reactor by routine methods in that state during the same period was $739. The federal expenditure for eradicating tuberculosis in animals during the fiscal year 1957 amounted to $1,807,868, and state legislatures appropriated $4,564,971 for the purpose during the same period. If all tuberculous infection evident at the time animals are slaughtered is reported and herds of origin promptly identified and tested, the efficiency of the program can be materially increased with the same expenditure of funds.

Although epidemiologic procedures produce a higher number of reactors in proportion to animals tested and are less costly per reactor, a great majority of the total reactors located in the eradication program are found as a result of testing for area and herd accreditation (table 1). Tracing procedures are a valuable adjunct to other eradication measures and deserve the increased attention they are getting, but they are not a substitute. Eradication officials believe that, with efficient tracing operations and more diligent application of other established procedures, the eradication of bovine tuberculosis will be hastened.

TUBERCULOSIS IN OTHER ANIMALS

Swine

Tuberculosis in other animals in the United States has declined along with bovine tuberculosis, although not to as great an extent. Losses of swine due to tuberculosis are shown in figure 6. Swine are susceptible to all three types of

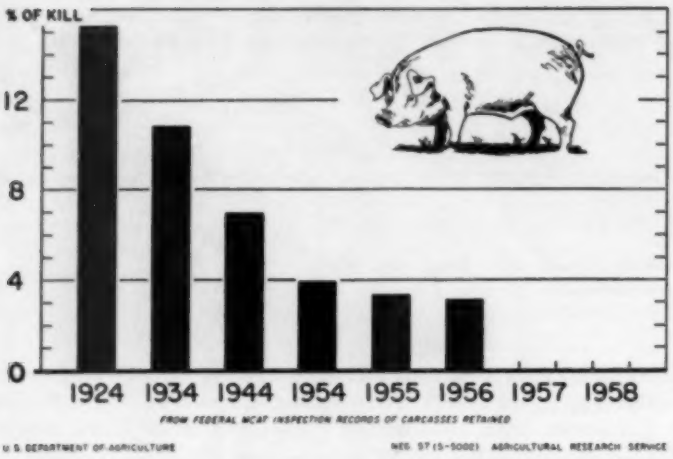


FIG. 6. Swine showing tuberculous lesions.

tuberculosis, human, bovine, and avian; but most of the tuberculosis recognized in swine today is avian, which is contracted from poultry. The bovine disease has practically disappeared in swine since tuberculosis has been reduced to a low level in cattle. Last year, however, tracing the origin of two lots of swine, in which 18 carcasses of 373 slaughtered animals were condemned, uncovered heavy infection in two herds of cattle. Tests on the 28 cattle on the farm from which some of the swine originated produced 21 reactors; 5 had extensive lesions. Tests on a herd of 29 cattle which a tenant had taken from the farm when he moved produced 17 reactors, only 2 of which failed to show lesions of tuberculosis on autopsy. The 18 head of breeding swine on the farm of origin were tested, and all reacted. The unusually large number of swine carcasses condemned on meat inspection and the severe infection found in the two herds of cattle suggest that in this case the swine were infected with bovine tuberculosis.

There have been no reports of swine infected with human tubercle bacilli in recent years. This may be because of the prohibition against feeding swine raw garbage, especially that originating from hospitals or sanatoriums.

Poultry

Poultry are susceptible only to avian tubercle bacilli. Although twenty or thirty years ago veterinarians noted extensive tuberculous infection in poultry, the disease has been reduced by improved management practices. Avian tuberculosis remains a problem in the North Central States where birds are carried over from year to year in laying flocks, and especially where farm flocks have the run of the premises. The disease is also found in wild birds in many parts of the country. Fortunately, tuberculosis is not a problem in the broiler industry because the birds are marketed at a young age.

When a flock is known to be infected, the recommended procedure is to raise a new flock on clean ground, and to dispose of old birds at the end of the laying season or when they are about eighteen months old. Because tuberculosis usually progresses slowly, most hens will not become a source of infection if they are handled in this manner. Careful attention to these and other management practices can be expected to result in the eradication of the disease from a flock.

Pheasants and turkeys are quite susceptible to avian tuberculosis. Ducks, geese, and swans are more resistant and, even when living with tuberculous chickens, they are seldom affected. Tuberculous lesions are frequently found in both captive and wild pigeons. In some parts of the world the condition occurs in natural epizootics among wood pigeons, not directly acquired from domestic fowl. Avian tuberculosis has been found to be common in starlings in England, as well as in crows, sparrows, and gulls.

Pets and Zoo Animals

Tuberculosis in dogs is rare in the United States. During the past two years only one report of canine tuberculosis has come to the attention of the Communicable Disease Center. It is not uncommon, however, in some parts of the world. According to a world survey, about 75 per cent of the strains isolated from naturally infected dogs are human, and nearly all of the remainder are bovine, with only a few avian strains. Although contaminated food may serve as a vehicle of infection, dogs usually acquire tuberculosis directly from man. Spread of infection from dog to dog or to other animals is very rare. The disease in dogs usually involves the thoracic organs, but may occur in the livers and kidneys. Lesions elsewhere are infrequent.

Cats are very resistant to human tuberculosis and seldom are infected by tuberculous owners. They are quite susceptible, however, to bovine infections, contracting the disease by drinking milk from tuberculous cows. In cats, the primary lesions are usually in the abdominal organs, with frequent secondary involvement in the lungs. As would be expected in view of the low incidence of bovine tuberculosis, there have been no reports of feline tuberculosis in the United States in recent years.

Interestingly enough, parrots in captivity are more susceptible to mammalian than to avian tubercle bacilli, and pet parrots may be infected with human tuberculosis. The lesions in parrots are most commonly on the head and appear as warty or horny growths on the root of the beak, nasal orifice, eyelids, and on the tongue. The internal organs are not always affected but, when they are, infection is usually confined to the lungs. Parrots infected with avian tuberculosis, however, show the characteristic nodules in the lungs, liver, and spleen.

Canaries are also susceptible to organisms of human tuberculosis but less so than they are to the avian strain. Human strains have been recovered from other pet birds, as well as wild birds.

While investigators have not found tuberculosis in monkeys living in the wild state, monkeys in captivity have an extremely high incidence of the disease. Tuberculosis can be a serious disease problem in the monkey colonies of lab-

oratories and zoos unless the animals are tuberculin tested frequently and precautions are taken to prevent the introduction of infection. The monkey is susceptible to both human and bovine infection, although most strains isolated are human strains. There can be little doubt that the monkey in captivity is the most susceptible of all animals to tuberculosis. There are about 100,000 monkey pets in the United States, according to the 1956 pet census made by *All Pets Magazine*, and every year a number of these animals are found to have tuberculosis.

In spite of their usefulness as laboratory animals, rabbits, guinea pigs, hamsters, mice, and rats rarely have natural tuberculous infection when they are kept as pets. Evidently tuberculosis does not occur in these animals in the wild state, except occasionally in mice and rats.

Wild animals not in contact with domestic animals or man are believed to be free of tuberculosis, but it is not uncommon in semi-wild or feral animals. For instance, foxes and minks raised on fur farms are quite susceptible to bovine infection, and animals kept in zoological parks are frequently infected. Minks are also susceptible to avian infection. It is of special interest that human tubercle bacilli, which are incapable of producing progressive tuberculosis in domestic cattle, goats, sheep, and pigs, can cause tuberculosis in the wild species of ruminants and pigs equal in severity to that produced in these wild animals by the bovine strain. There are 8 recorded cases of fatal tuberculosis in elephants confirmed by autopsy and bacteriologic examination; all yielded human tubercle bacilli.

Bovine Tuberculosis in Other Countries

The situation in the United States with regard to tuberculosis in cattle is better than that of most of the rest of the world. The only area that has a lower incidence than the United States is the Scandinavian countries. Finland, Sweden, Norway, and Denmark have almost completely eradicated bovine tuberculosis; in these countries now, tuberculin reactions in cattle are chiefly due to exposure to human infection.

Another encouraging report comes from Holland which has reduced the incidence of tuberculosis among animals to less than 1 per cent. Dutch veterinarians and public health workers realized early that agricultural workers, rural residents, and others having contact with cattle should be checked for tuberculosis. In their control campaign, they are testing both animals and humans.

Germany has made considerable progress during the past decade, influenced by United States Army and Air Force veterinarians who supervised the procurement of milk supplies for the troops stationed there. The Germans have recently enlarged their program and they hope to eliminate bovine tuberculosis within the next decade.

France has also had the stimulus of United States procurement requirements in some areas and a number of "islands" of disease-free animals have been established. For forty years the French used BCG as an immunizing agent for cattle, but they discontinued this program early in 1955, and now plan to carry on a national testing and slaughter program.

England, Scotland, Wales, and Northern Ireland are moving along toward eradication of tuberculosis in cattle. Recent reports state that 85 per cent of the herds in England are known to be disease-free and a sizeable number of the remaining herds probably are as well. The dramatic drop in the incidence of bovine tuberculosis among children in England attests to the public health significance of the program.

Although Portugal is almost free of bovine tuberculosis in cattle, Spain and Italy have the highest incidence of the disease in cattle in Western Europe. Italy has depended upon BCG vaccine for many decades and is still continuing its use, especially in northern Italy, which is known to have a high incidence of bovine tuberculosis.

During the past few years reports have come from Italian investigators on the use of isoniazid in the treatment of animal tuberculosis. According to early information, the results are equivocal. Further data received early in 1957 indicate that drugs are of little or no value in eliminating tuberculosis from cattle and that maintaining the necessary levels of drugs is uneconomical. It is also reported that, as soon as the drugs are withdrawn, the disease becomes active.

Information is limited on either the prevalence of tuberculosis in cattle or steps taken to control it in the countries of Eastern Europe. The disease is known to be present in Africa, especially in European breeds of cattle, and in the dairy centers in Asia.

Australia and New Zealand have a low incidence, and both have inaugurated eradication programs. With their well-organized veterinary services, they should be able to wipe out the disease in the next decade.

Outside the United States and Canada, which has many areas with no tuberculous cattle, the problem is still of major concern in the Western Hemisphere. Tuberculosis in cattle is common in the milk sheds of populous centers in South America, and is even to be found in beef animals of the Argentine. Only Venezuela and Colombia have eradication campaigns, and in the latter country the program has been inactive in recent years.

Bovine tuberculosis can be brought under control and eventually eradicated when public health and veterinary medical officials receive the support of the public and of legislative groups. This support is the keystone to control of the disease, and it can be developed when there is an enlightened public and the necessary leadership among public health and veterinary authorities.

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A SIMPLE METHOD FOR REDUCING THE RADIATION HAZARD ASSOCIATED WITH PHOTOFLUOROGRAPHY[1]

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(Received for publication October 24, 1957)

In view of the wide publicity recently given to the potential dangers from X-radiation, and particularly to the statements of the National Academy of Sciences regarding possible genetic effects, considerable thought is being given to methods of reducing the radiation involved in diagnostic roentgenology. Inasmuch as photofluorographic examinations constitute a large proportion of all roentgenographic examinations of the chest, a review of the techniques used in this type of examination seems indicated in an effort to develop changes in technique which will reduce unnecessary radiation. It is the purpose of this paper to report some methods which are simple to apply to photofluorographic equipment and which will reduce radiation exposure.

Material and Methods

For this purpose a phantom was made of untempered masonite which had about the same density and radiation-absorbing qualities as human tissue (figure 1). The absorption qualities of this phantom were checked with a step-wedge of the masonite and were found to be quite similar to that of water. To determine the ratio of phantom to chest thickness, a phototimer and milliampere-second meter were used. A human subject with an anteroposterior chest thickness of 21 cm. was placed between the tube and phototimer, and the required exposure for a film of the proper density was determined. Then by trial and error, the equivalent thickness of masonite was determined which would produce the same film density with the same setting of the machine. The equivalent thickness of masonite was found to be approximately five-eighths of the measured anteroposterior thickness of the chest. Therefore, the phantom was constructed to a five-eighth scale to simulate the absorption quality of the average chest in the anteroposterior plane, and to full scale in the lateral dimensions, to obtain measurements of the effects of internal body-scatter.

Estimates of the radiation exposure to the reproductive organs were made with Victoreen and Keleket dosimeters with a maximal sensitivity of 0.2 roentgen (r) and a minimal sensitivity of 1.0 milliroentgen (mr.). Estimates of skin dosage were made with Bendix dosimeters with values given in mean dose per exposure, the maximal sensitivity being 50 r and the minimal sensitivity 1 r. All dosimeters were calibrated shortly before the measurements were made.

The phantom was placed in front of the photofluorographic hood with two dosimeters on the surface toward the tube and two on the opposite surface. Two additional dosimeters were placed in the male gonadal area. The target-hood distance was always kept at exactly 42 inches.

Several hundred exposures were made comparing the factors involved. All measurements of radiation of the reproductive glands were made in the male gonadal area. The X-ray generator used was a General Electric Maxicon rated at 200 milliamperes (ma) at 100 kilovolt peak (KVP). The tube was a General Electric rotating-anode, double focus 1.0 mm.

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[1] From the Division of Tuberculosis Control, New York State Department of Health, Albany, New York.

FIG. 1. The phantom of masonite.

and 2.0 mm. All tests were made using the small focal spot. The hood was equipped with a Patterson E-2 green screen and a curved grid with an 8:1 ratio focused at 42 inches.

The effect of the following was studied:

(*1*) The changes in the shape and size of the diaphragmatic opening at the tube

(*2*) The effect of local shielding

(*3*) The effect of filtration of the X-ray beam

(*1*) In order to determine the importance of the proper diaphragm in limiting X-ray exposure, a lead diaphragm, one-eighth inch thick, was used at the X-ray tube port to limit

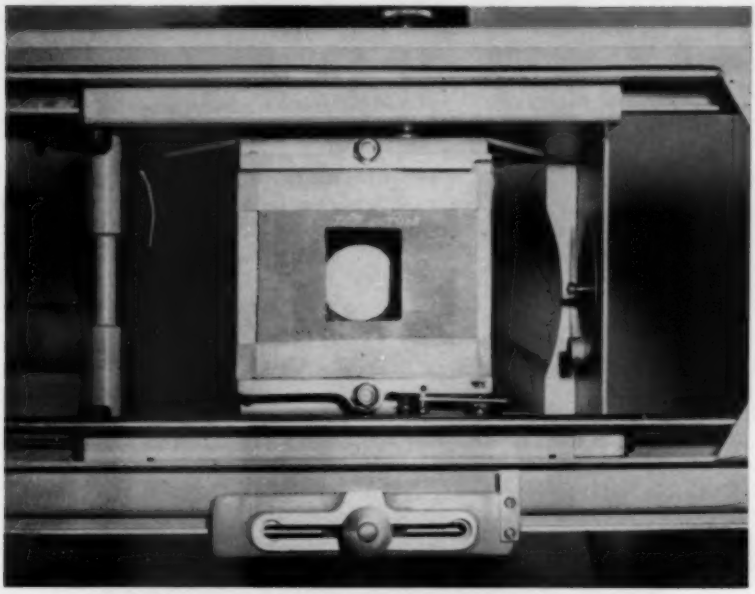

FIG. 2. Photograph of the lead diaphragm used at the tube port.

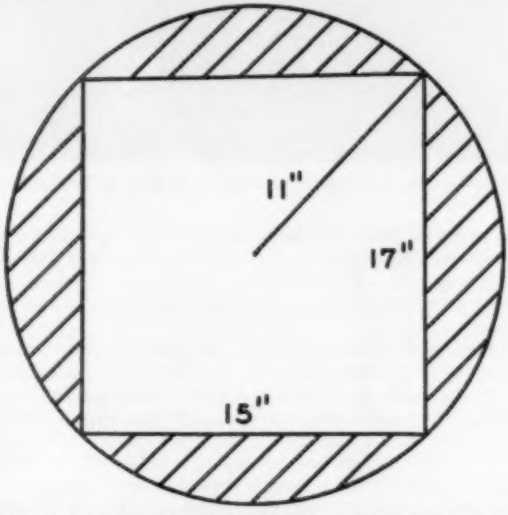

FIG. 3. Comparison of circular cone and diaphragm. The hood is a rectangle 15 by 17 inches, and the circular area to cover this rectangle must have a radius of at least 11 inches. The area of the hood is 255 square inches, and the smallest area covered by a circular cone that includes the hood-front is 397.94 square inches. Therefore, with the use of a circular cone, almost 40 per cent of the primary beam goes over, under, and alongside the hood to cause extra unnecessary and unwanted scatter radiation.

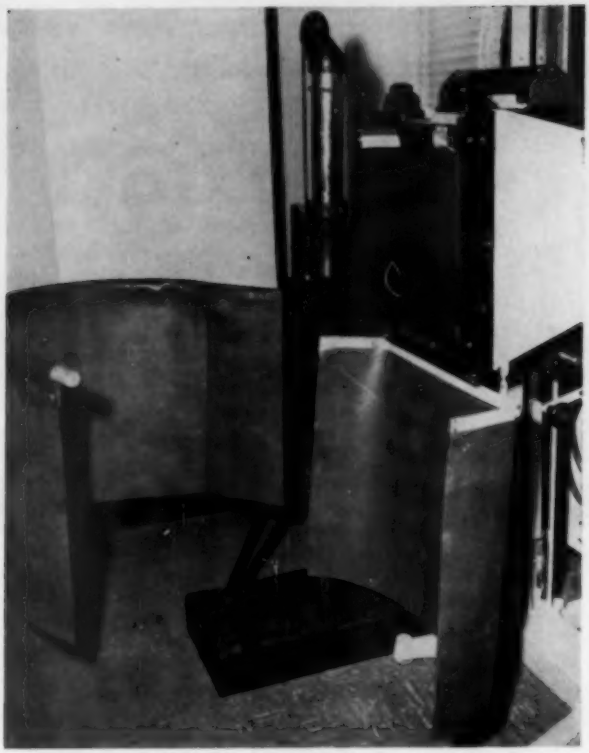

FIG. 4. Photograph of leaded rubber shielding with hinged bracket open.

the size of the radiated area (figure 2). The shape and size of the collimating device is critical. In order to make sure that the primary beam was contained to the hood front the following adjustments were made: The target-hood distance was always kept at exactly 42 inches and a fluorescent screen, the size of the hood-front, was attached to the front of the hood.

The opening of the lead diaphragm was cut, a small amount at a time. Often during this procedure the room was dimmed and an X-ray exposure was made against the fluorescent screen to see the actual area irradiated. The diaphragm opening of proper size was attained when there was a border of unilluminated screen 1 inch wide around the edge of the screen.

Such a device is better than a square cone since the opening of the cone cannot be adjusted, and the only way to limit the primary beam to the hood is by adjusting the target-hood distance unless a specially made cone is obtained for the required hood distance. A circular cone or diaphragm for this type of roentgenographic examination is not recommended. It is better than no collimating device at all, but it has the serious disadvantage of allowing a large portion of the primary beam to spread out beyond the hood (figure 3). Clarke (1) has emphasized and illustrated the importance of this factor in reducing unwanted radiation.

(*2*) Local shielding was provided by the use of lead rubber sheeting hung below the front of the hood. This extended approximately 10 inches beyond the hood on each side and

FIG. 5. Same as figure 4 with bracket in place, ready for X-ray exposure.

extended down a distance of 24 inches. Another segment of lead rubber was hung on a hinged bracket in such a way as to allow the patient to be positioned when the bracket is closed so that the lower portion of the body is completely encircled (figures 4 and 5). This sheeting offered X-ray protection equivalent to 0.5 mm. of pure lead.

(*3*) Adequate filtration of the beam at the tube port was provided in the form of 2.0 mm. of aluminum added to the inherent filtration of the glass, which in this case was equivalent to 0.5 mm. of aluminum.

The highest kilovoltage permissible within the safety range of the tube was used in order to keep the radiation dosage to a minimum.

RESULTS

The results of this study are shown in table 1, which gives the total readings resulting from 50 exposures. The amount of radiation exposure received by the phantom when no protective devices were used is shown in the first column of the table. The effect of the lead diaphragm in reducing the amount of exposure is shown in column 2. As may be seen, the amount of radiation to the gonadal area is reduced to less than one-fifth simply by the use of a proper lead diaphragm at

TABLE 1

EFFECT OF THREE PROTECTIVE DEVICES IN REDUCING RADIATION TO THE PATIENT

Area Monitored	(1) No Lead Diaphragm No Lead Rubber No Filters	(2) With Lead Diaphragm No Lead Rubber No Filters	(3) With Lead Diaphragm With Lead Rubber No Filters	(4) With Lead Diaphragm With Lead Rubber With 2.0 mm. Added Filtration
Gonadal area of male patient.....	170.0 mr	30.0 mr	22.0 mr	20.0 mr
Skin dose (or entrance dose) with back-scatter.....	45.2 r	37.5 r	37.5 r	25.0 r
Exit dose.....	4.0 r	3.8 r	3.8 r	3.8 r

the tube. Also it reduces the skin dose by about 20 per cent. For the maximal efficiency of this diaphragm, its shape should be rectangular since the hood is also rectangular in shape.

Column 3 of the table shows the added protection afforded by local shielding. Although this effect is not as marked as that of the use of a proper diaphragm, nevertheless, it still results in further reduction to the gonads.

The value of aluminum filtration of the X-ray beam is shown in column 4. While there is only a slight reduction in radiation of the gonads, the main value of this filtration consists in a reduction of the skin dose by about one-third.

DISCUSSION

Data used by Perkins (2) show that the average radiation dose to the gonads resulting from the production of a conventional 14- by 17-inch chest roentgenographic film is approximately 0.00025 r and that from a conventional photofluorogram is approximately 0.005 r. These data were obtained from a study at the Brookhaven National Laboratory (3), in which calculations were made on the exposures incident to some common diagnostic procedures using only 0.5 mm. aluminum as a filter at the tube port. If the total permissible gonadal dose from all sources of radiation from conception to age thirty is 10 r (4), it has been calculated that yearly photofluorograms from age fifteen to age thirty would use up less than 1 per cent of this permissible dose of 10 r. In spite of this wide safety factor, however, since the effect of all ionizing radiation is cumulative and since all unnecessary radiation should not be permitted, further reduction of exposure is warranted.

Considerable thought should be given to the relative value of diagnostic roentgenographic procedures as compared with the radiation hazard involved during these procedures, so that when the expected results of diagnostic procedures are questionable they should not be used.

Furthermore, another factor, not considered in detail in this paper but of considerable importance, is the advisability of using the fastest X-ray film and screen combinations available. At present the green fluorescent screen and green sensitive films present the best combination for photofluorography.

Finally, the proper use of the protective devices and techniques considered above will result in a considerable reduction of the amount of radiation involved in the use of photofluorographic equipment.

Summary

In order to test the effectiveness of certain devices designed to reduce the radiation hazards associated with photofluorography, a masonite phantom was constructed which had approximately the same density as human tissue. Estimates of radiation exposure to the gonads and to the skin were made with appropriately placed dosimeters. Several hundred exposures were made to test the effect of (*1*) a proper lead diaphragm at the tube port to confine the primary beam to the desired area; (*2*) local shielding of the lower half of the examinee's body by leaded rubber; and (*3*) additional filtration of the X-ray beam. It was found that the use of these three simple devices resulted in a tenfold reduction in radiation received by the gonads, and a 50 per cent reduction in the skin dose. Since the radiation associated with photofluorography is considerably higher than that from conventional roentgenography of the chest, it is mandatory that photofluorographic X-ray installations be properly shielded.

The methods outlined above afford a simple and inexpensive method for attaining this objective.

Sumario

Sencillo Método para Reducir la Asociación de Riesgo Irradiatorio con la Roentgenofotografía

A fin de comprobar la eficacia de ciertos medios destinados a reducir los riesgos de la irradiación, vinculados con la roentgenofotografía, se construyó un fantasma de masonita que poseía aproximadamente la misma densidad que el tejido humano. Con dosímetros colocados apropiadamente se hicieron cómputos de la exposición de los gonados y la piel a la irradiación. Se llevaron a cabo varios centenares de exposiciones para determinar el efecto de (1) un diafragma apropiado de plomo en la puerta del tubo para limitar el haz primario a la zona en mira; (2) resguardo local de la mitad inferior del cuerpo del sujeto con caucho plomizo; y (3) mayor filtración del haz de rayos X. Se descubrió que el empleo de esos tres sencillos medios daba por resultado una reducción al décimo de la radiación recibida por los gonados y una reducción de 50 por ciento en la dosis cutánea. Como la radiación vinculada con la roentgenofotografía es considerablemente más alta que la de la roentgenografía habitual del tórax, es imperativo que es resguarden debidamente las instalaciones de rayos X destinadas a la roentgenofotografía.

Los métodos expuestos más arriba ofrecen una técnica sencilla y poco costosa para alcanzar este objetivo.

Resume

Une méthode simple pour réduire les dangers de l'irradiation associée á la photoradiographie

De manière à éprouver l'efficacité de certains appareils destinés à réduire les dangers de l'irradiation en photoradiographie, un corps artificiel en masonite, ayant approximativement la même densité que les tissus humains, a été construit. L'évaluation de la dose de rayons à laguelle les gonades et la peau étaient exposés a été faite avec des dosimètres placés de façon appropriée. Plusieurs centaines d'expositions aux rayons ont été faites pour éprouver l'effet des mesures suivantes: 1) un diaphragme en plomb approprié placé à l'orifice du tube pour confiner le rayon primaire à une région déterminée; 2) protection locale de la partie

inférieure du corps du sujet avec du caoutchouc plombé; 3) filtration additionnelle du faisceau de rayons X. On a constaté que l'usage de ces trois simples mesures réduisait de dix fois la dose de rayons reçus par les gonades et de 50% celle de la peau. Comme l'irradiation en photoradiographie est considérablement plus élevée que celle de la radiographie conventionnelle des poumons, il est indispensable que des écrans protecteurs adéquats soient prévus dans les installations destinées à radioscopie.

Les méthodes mentionnées ci-dessus représentent un moyen simple et peu couteux d'atteindre cet objectif.

Acknowledgment

The authors wish to acknowledge the technical assistance of Mr. Frank Litto and Mr. Cecil Edwards in the construction of the phantom and the lead rubber shielding.

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OCCULT TUBERCULOUS ENDOBRONCHITIS IN SURGICALLY RESECTED LUNG SPECIMENS[1]

J. ROBERT THOMPSON AND G. KENT

(Received for publication October 28, 1957)

INTRODUCTION

Since the advent of the antimicrobial agents the incidence of tuberculous lesions in the bronchi as seen bronchoscopically has markedly decreased. The lack of epithelial lesions, however, does not negate the possibility of there being active submucosal tubercles. Because of this possibility of hidden or occult tuberculous foci, it was thought that it would be of interest to study in detail the histopathologic changes in the bronchi which communicated with cavitary lesions or with those bronchi which drained the area in which solid lesions were found.

PROCEDURE

The pulmonary tissue (exclusive of wedges) surgically resected from 344 patients consecutively during 1955 and 1956 was studied. Blocks of tissue were taken from three areas: (*1*) the juncture of the bronchus with the parenchymal lesion; (*2*) the stump or point of amputation of the bronchus or branch bronchi; and (*3*) a point midway between the point of amputation and the tuberculous lesion. Sections were made at three different levels of each block and were stained with hematoxylin and eosin and Ziehl-Neelsen stains. Only those sections which showed evidence of tubercle formation were classified as tuberculous endobronchitis. Bacteriology studies consisted of smearing and culturing material aspirated from the bronchial tree or, in the event no secretions were present, flushing the tree with sterile saline.

OBSERVATIONS

The 44 cases of tuberculous endobronchitis were found in the resected tissue from 11 pneumonectomies, 26 lobectomies, and 7 segmentectomies. There were 24 women and 20 men in the series and of these 21 were white, and 23 were non-white. The youngest patient was fourteen years of age and the oldest sixty-four years of age, the greatest number being in the twenty- to thirty-year age group (34.5 per cent). All of the patients had been on combined chemotherapy for various periods of time prior to surgery and, in addition, many had received pneumoperitoneum or some form of collapse therapy prior to resection.

When the resected specimens were examined grossly, some alteration of the bronchial mucosa was noted in 6 cases. Histologic evidence of tuberculous endobronchitis was found in 44 cases (12.8 per cent). The distribution of the lesions was as follows: at the stump (area 2) in 15 cases; mid-way between the stump and parenchymal lesion (area 3) in 18 cases; in both areas 2 and 3 in 11 cases; and adjacent to the parenchymal lesion (area 1) in all cases.

The peribronchial lymph nodes were studied, and evidence of active disease

[1] From the Laboratory of the City of Chicago Municipal Tuberculosis Sanitarium.

TABLE 1

INCIDENCE OF CAVITATION, TUBERCULOUS LYMPHADENITIS, AND THE ISOLATION OF TUBERCLE BACILLI IN 344 CONSECUTIVE RESECTIONS (EXCLUSIVE OF WEDGES)

	Tuberculous Endobronchitis 44 Cases		No Tuberculous Endobronchitis, 300 Cases	
	Number of Cases	Per Cent	Number of Cases	Per Cent
Cavitation	40	90.9	103	34.3
Lymphadenitis	6	14.4	6	2.0
Cultures positive for tubercle bacilli	25	64.0*	13	6.5†

* 39 specimens cultured.

† 199 specimens cultured.

was found in only six (2.0 per cent) of the nontuberculous endobronchitis group whereas the incidence was much higher (14.4 per cent) in the cases of endobronchial disease. This suggests the possibility that the bronchial lesions might be the result of extension of the disease through the peribronchial lymphatics. In the nontuberculous endobronchitis group, two of the six specimens showed cavitation, and the remaining four did not show any evidence of cavitary disease.

Material which was aspirated from the bronchi in the tuberculous endobronchitis group yielded a high incidence of tubercle bacilli on culture (64 per cent) as compared with the relatively low percentage in the nontuberculous-endobronchitis group (6.5 per cent).

DISCUSSION

The incidence of tuberculous endobronchitis has decreased markedly since the advent of the antimicrobial agents. For example, in 1945 Overholt and Wilson (1) reported 60 patients with endobronchial disease, one-third of whom had bronchoscopic evidence of tuberculous tracheobronchitis preoperatively. In autopsy material from untreated persons, the incidence of tuberculous tracheobronchitis was even higher. In all of the 44 cases reported in this series, preoperative endoscopy was essentially normal; however, 10 of the patients had shown some evidence of endobronchial disease on previous examination.

Consideration of the pathogenesis and pathology of tuberculous endobronchitis are contingent upon an understanding of the anatomy of the bronchus. There are numerous crypts in the bronchial muscosa which communicate with the ducts of the seromucinous glands. In addition there are numerous lymphatics within the lung, but those pertinent to the present discussion are the lymphatics of the bronchi. The larger bronchi have two sets of lymphatics which are so arranged that they enclose the cartilages and connect with each other by anastomosing vessels which pass between the cartilaginous plates. The smaller ramifications of the bronchial tree have only a single plexus. Likewise, there is abundant pulmonary lymphoid tissue which is present about the bronchi, arteries, and veins, and may occur in the form of lymph nodes, lymph follicles, or small masses of lymphoid tissue.

FIG. 1. Gross photograph of a lung depicting the areas from which tissue blocks were taken.

Most writers contend that there are three possible pathogenetic factors in the development of tuberculous endobronchitis. The first, and by far the commonest, is surface infection due to contact with bacilli-laden secretions; second, lymphatic extension from a peribronchial focus; and, third, contiguous invasion of the bronchial mucosa. In a discussion of tuberculous tracheobronchitis by Bugher and associates (2), it is pointed out that the bronchial lesions are similar to tracheal lesions in which there is no evidence of any peritracheal infection nor is there any disease in the peritracheal lymph nodes. Their conclusion was that the tubercles present in the wall of the trachea are the result of surface implantation through the glandular ducts. These lesions likewise are unrelated to any hematogenous dissemination. Sweany and Behm (3) favor the direct implantation mechanism as the most likely pathogenesis of tuberculous endobronchitis, citing tuberculous enteritis as a comparable disease process. There was no evidence in their series of cases to support "the 'back-door' entrance of germs through the mucosa because the structure in and outside of the adventitious layers were free of disease." On the other hand, Schwartz (4) collected a large number of cases

FIG. 2. Section of a bronchus showing two subepithelial tubercles: The larger has a central caseous zone and is well demarcated while the smaller (in the redundant mucosa) is cellular and less well defined. The bronchial epithelium is intact throughout but in some areas metaplastic change is noted. (Hematoxylin and eosin stain, × 60)

in which he demonstrated perforations of tuberculous lymph nodes into the bronchial tree. He contended that lymph node perforation was the major pathogenetic factor in chronic tuberculosis. In addition, he pointed out that the mediastinal lymph node bridge permits passage of infection from one lung to the other.

The majority of lesions in the present series were situated within or adjacent to seromucinous glands, and in only a few instances was there any doubt of the pathogenesis (whether the lesion was the result of a seeding from the mucosal surface or whether it might be lymphogenous in origin). The fact that 40 of the 44 specimens also contained a cavity and because of the large number of instances in which tubercle bacilli were cultured (64.0 per cent), it seems that in the majority of instances the endobronchial disease was the result of surface implantation of bacilli. However, the incidence of lymph node involvement (14.4 per cent) bespeaks the possibility of lymphatic extension as a cause of tuberculous endobronchitis.

It is not surprising that normal bronchoscopy findings were reported. It has been shown previously, especially in tuberculous laryngitis (5), that, with antimicrobial therapy, there is rapid healing or repair of an epithelial surface. Hardy and Samson (6) described a "quiescent" bronchus which was essentially negative on endoscopic examination but was "not a healed bronchus in a histopathologic sense." In a review of 602 resected specimens, Olson and co-workers (7) reported that 37.9 per cent of the patients with normal bronchoscopic findings showed evidence of endobronchial disease on microscopic examination of the resected

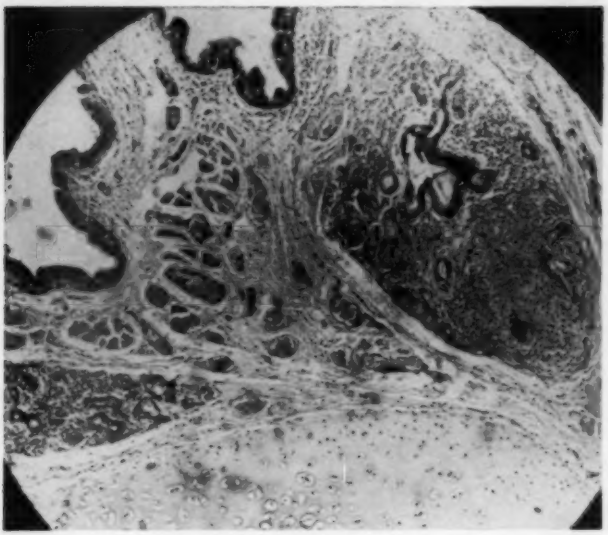

FIG. 3A.

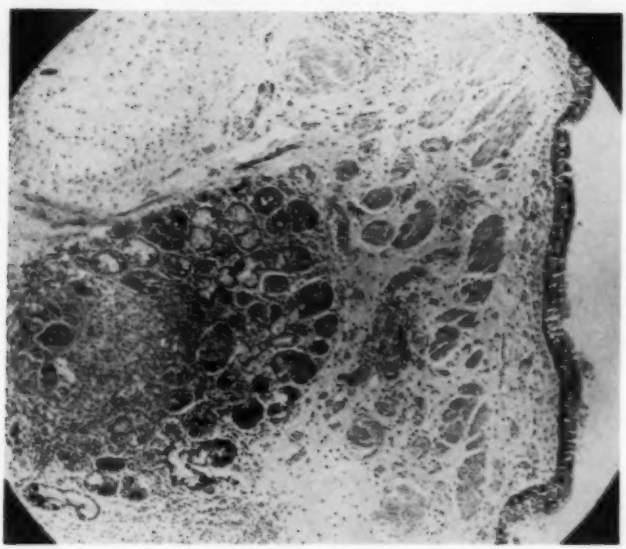

FIG. 3B.

FIG. 3A and B. Two photomicrographs to illustrate tubercles in the seromucinous glands. The bronchial epithelium is normal in appearance, and there is little reaction in the surrounding submucosal structures. (Hematoxylin and eosin stain, × 60)

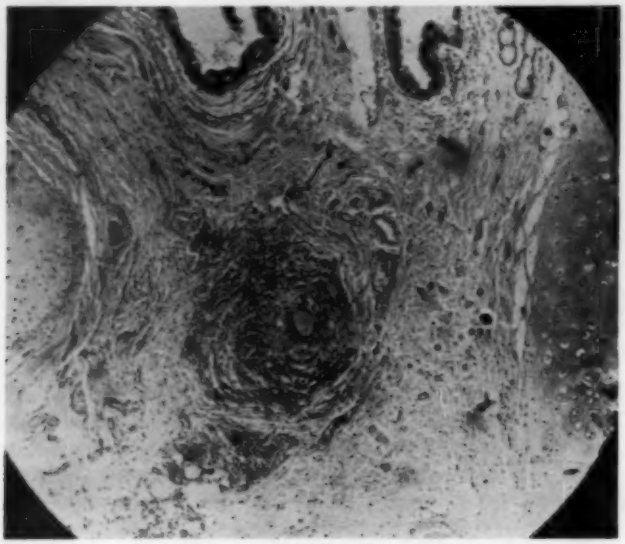

FIG. 4A.

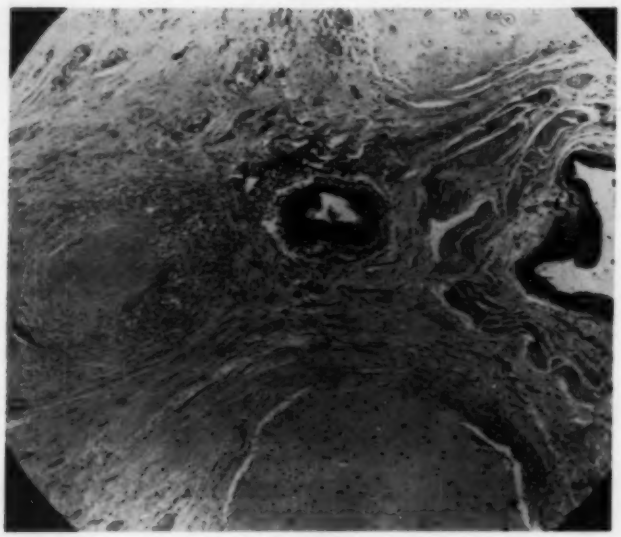

FIG. 4B.

FIG. 4A and B. Two submucosal tubercles are shown here. No glandular elements are seen in either lesion suggesting involvement of peribronchial lymphatics rather than surface implantation into glands. There is considerable fibrous reaction about the older lesion (B). (Hematoxylin and eosin stain, × 60)

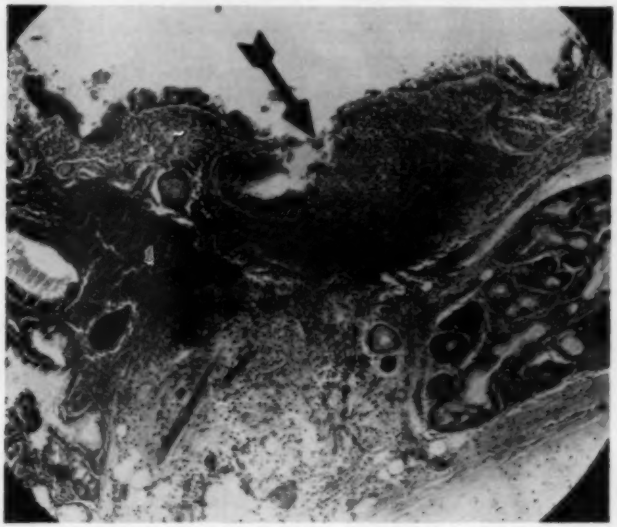

FIG. 5. Photomicrograph showing ulceration (arrow) of the bronchial mucosa due to a superficial tubercle. There is marked infiltration of the submucosa with chronic inflammatory cells. (Hematoxylin and eosin stain, × 60)

specimens. In a series of 100 resected specimens, Andrews and co-workers (8) found that 16 showed histologically normal bronchi, 66 showed marked lymphocytic infiltration of the bronchial wall, and 18 contained typical tubercles. Undoubtedly many of the specimens showing only the nonspecific inflammatory changes represent a healed or healing tuberculous infection. There is a possibility however, that the bronchiectatic changes noted are as much the result of parenchymatous changes as they are of previous endobronchial tuberculosis. In this same series of 100 cases, 23 per cent of the patients with lymphocytic infiltration developed postoperative complications as compared with 22 per cent of the patients with submucosal tubercles, emphasizing the fact that, even in the absence of typical tuberculous lesions, complications are just as likely to occur.

What is the significance of these occult tuberculous lesions in the bronchi? Perhaps the most dreaded complication of tuberculous endobronchitis is the postoperative development of a bronchopleural fistula due to the breaking down of the stump. Two bronchopleural fistulas developed in the 44 cases of endobronchitis, and in both instances the lesions were too small to be recognized grossly. A second significant factor is that cases of occult tuberculous endobronchitis do give a high incidence of cultures positive for tubercle bacilli of the material aspirated from the bronchus. While the most likely source of the bacilli is the cavity communicating with the bronchus, the fact that no cavity was present in some specimens yielding a culture positive for tubercle bacilli leads one to conclude that a microscopic endobronchial lesion is capable of discharging sufficient tubercle bacilli to be demonstrated on culture.

Would prolonged chemotherapy completely eradicate the disease? Andrews and associates (8) reported a higher incidence of postoperative complications (23 per cent) in patients who had been on chemotherapy for a period exceeding eight months than in the group with treatment for less than eight months (15 per cent). Undoubtedly the first group of patients had more severe pulmonary disease. It has been shown by several workers that very often an open cavity yielded tubercle bacilli which grew rapidly but were resistant to drugs, whereas the closed cavity in which little drug effect was likely to be displayed consistently yielded bacilli which required prolonged incubation. If viable tubercle bacilli remain within a cavity despite long-term chemotherapy, then the bronchial tree is constantly contaminated and the possibility is very great that a tuberculous endobronchitis will ensue and/or persist. Suffice it to say that one cannot predict with certainty whether prolonged chemotherapy would have eradicated the tuberculous foci noted in the bronchial walls of the surgical specimens.

The incidence of endobronchial lesions in the resected specimens is comparable with that seen in the intestinal tract in cases of pulmonary tuberculosis examined at autopsy. In this situation also, there are mucosal lesions which are the result of constant contamination of the surfaces with viable tubercle bacilli. Compared with the incidence of mucosal lesions prior to chemotherapy, the reduction in the incidence of these lesions has been spectacular. In the light of the present study, it can be anticipated that residual lesions will persist within the bronchial walls of some patients whose disease is refractory to treatment, and such cases will continue to pose a problem for the clinician.

Summary

A study of the histopathologic changes in the surgically resected pulmonary tissue from 344 consecutively treated patients with tuberculosis is presented. Preoperative endoscopic examination failed to reveal the presence of any tuberculous bronchial lesions. Blocks of tissue were taken to include the bronchus which drained the principal parenchymal lesion at three different levels: (*1*) the juncture of the bronchus with the parenchymal lesion, (*2*) the stump or point of amputation of the bronchus or branch bronchi, and (*3*) a point midway between the stump and the tuberculous lesion.

Prior to fixation of the specimens, material was aspirated from the bronchial tree for bacteriologic study. Histologic evidence of tuberculous endobronchitis was found in 12.8 per cent of the patients, and 14.4 per cent of this group showed evidence of active disease in the peribronchial lymph nodes as compared with only 2 per cent with lymph node involvement in the nontuberculous endobronchitis group. Cultures positive for tubercle bacilli were obtained in 64 per cent of the endobronchitis group compared with only 6.5 per cent in the group without endobronchitis.

Sumario

Endobronquitis Tuberculosa Oculta en Ejemplares Pulmonares Resecados Quirúrgicamente

Preséntase un estudio de las alteraciones histopatológicas en el tejido pulmonar resecado quirúrgicamente a 344 enfermos tratados consecutivamente. El examen endoscópico pre-

operatorio no reveló la presencia de lesiones bronquiales. Se tomaron bloques de tejido que comprendían el bronquio que drenaba la principal lesión parenquimatosa a tres niveles distintos: la unión del bronquio con la lesión parenquimatosa, el muñón o punto de amputación del bronquio o de las ramas bronquiales y un punto intermedio entre el muñón y la lesión tuberculosa. Antes de fijar los ejemplares, se aspiró material del árbol bronquial para estudio bacteriológico. En 12.8 por ciento de los enfermos se encontraron signos histológicos de endobronquitis tuberculosa y 14.4 por ciento de este grupo revelaron signos de enfermedad activa en los ganglios linfáticos peribronquiales, comparado con 2 por ciento solamente que tenían invasión de los ganglios linfáticos en el grupo de endobronquitis no tuberculosa. Se obtuvieron cultivos positivos para bacilos tuberculosos en el grupo de endobronquitis, comparado con no más de 6.5 por ciento en el grupo sin endobronquitis.

Resume

Endobronchite tuberculeuse occulte dans des pièces d'exérèse pulmonaire

Présentation d'une étude relative aux modifications histopathologiques observées dans les tissus pulmonaires réséqués dans une série de 344 cas consécutifs. L'examen endoscopique préopératoire n'avait pas révélé la présence de lésions bronchiques. Des blocs de tissu comprenant la bronche de drainage de la lésion parenchymateuse principale ont été prélevés à trois niveaux différents: à la jonction de la bronche et de la lésion du parenchyme, au moignon ou point d'amputation de la bronche ou des ramifications bronchiques et en un point intermédiaire entre le moignon et la lésion tuberculeuse. Avant la fixation des pièces, les sécrétions étaient aspirées à partir de l'arbre bronchique pour étude bactériologique. Des signes évidents d'endobronchite tuberculeuse ont été découverts chez 12.8 pour 100 des malades et, dans ce groupe, 14.4 pour 100 présentaient des signes de tuberculose en activité dans les ganglions péribronchiques; en comparaison, dans le groupe avec endobronchite non tuberculeuse les ganglions lymphatiques n'étaient intéressés que dans 2 pour 100 des cas. Des cultures positives pour le bacille tuberculeux ont été obtenues dans 64 pour 100 des cas dans le groupe atteint d'endobronchite par comparaison avec 6.5 pour 100 seulement dans le groupe sans endobronchite.

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THE EFFECTS OF CHEMOTHERAPY UPON THE TUBERCULIN SKIN REACTION IN GUINEA PIGS INFECTED WITH THE H37RV STRAIN OF TUBERCLE BACILLI[1, 2, 3]

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(Received for publication September 9, 1957)

Introduction

The validity of the tuberculin skin test as a means of determining the infection rate of *M. tuberculosis* in humans and in animals has seldom been seriously questioned as is attested by the long history of its widespread use. The literature relating to this subject is extensive, and any study of its reliability at this time may seem redundant. The availability of chemotherapy has wrought such marked changes in the course of tuberculosis, however, that it seems proper to study again some of the fundamental concepts of the host-parasite relationships in the light of these changes.

Prior to the use of drug therapy, a definitely positive tuberculin skin test resulting from a naturally acquired tuberculous infection was infrequently observed to revert spontaneously to negative. When reversion did occur, except under very well-recognized circumstances, it was generally considered to be good evidence that a biologic cure had occurred. This loss of tuberculin skin allergy in man was also generally assumed to be accompanied by the loss of acquired immunity (1) although a different interpretation is suggested by certain animal experiments. In animal studies, Willis (2) has suggested that specific immunity persists in tuberculous infection even though a previously positive tuberculin skin reaction has reverted spontaneously to negative.

The reversion to negative of positive tuberculin skin tests in humans (3, 4) and in animals (5, 6, 7) receiving chemotherapy for tuberculous infections has been observed. The question of whether this kind of a negative tuberculin skin test carries the same connotations as a negative tuberculin skin test observed in an individual who had never been infected with tubercle bacilli, and the frequency of its occurrence, should be clarified. The effectiveness of chemotherapy has increased the hope of biologic cure for certain types of tuberculous infection. Reliable tests for substantiating such achievements would be desirable, and the effect of the eradication of the infection upon the allergy-immunity mechanism of the host would increase the basic understanding of the pathogenesis of tuberculosis.

The present experiment was designed to study the effects of chemotherapy

[1] From the Laboratory Department, McCain Division of the North Carolina Sanatorium System, McCain, North Carolina.

[2] Presented in part before the Southern Trudeau Society, annual meeting, October 3, 1956, Dallas, Texas.

[3] This study is supported by a research grant from the Gustavus and Louise Pfeiffer Foundation, New York.

upon the tuberculin skin reaction and the possible correlation of acquired immunity and the behavior of the tuberculin skin reaction in guinea pigs treated with prolonged multiple-drug antituberculous therapy. This study is concerned primarily with the allergic aspect of the problem. The question of acquired immunity and its relationship to allergy is being investigated in another study.

Materials and Methods

Sixty-seven tuberculin-negative albino guinea pigs weighing approximately 400 gm. each were inoculated subcutaneously in the region of the sternum with approximately 800,000 viable units of the strain H37Rv. These animals were then randomly divided into two major groups:

Group 1, composed of 22 animals, received antimicrobial therapy beginning at the time of inoculation. *Group 2*, composed of 45 animals, also received antimicrobial therapy beginning sixteen days after inoculation.

The medication in both groups consisted of several multiple-drug regimens, all of which included isoniazid.[4] Other drugs used in various regimens with isoniazid included streptomycin, pyrazinamide,[5] and para-aminosalicylic acid (PAS). Isoniazid, 10 mg. per animal per day, and streptomycin, 25 mg. daily, were administered by subcutaneous injection; pyrazinamide and PAS were administered in ground commercial laboratory "chow" in 1 per cent and 2 per cent concentrations, respectively. The animals received the latter two drugs at all times. Chemotherapy was administered seven days a week to all of the surviving animals for a period of six months. This was followed by a six-month period of observation without chemotherapy.

Tuberculin skin tests were performed on the eleventh, thirteenth, and sixteenth days after inoculation in all animals which reacted negatively to the initial or any one of the previous tests. The tests were repeated every two weeks for the next six months, and infrequently (usually at monthly intervals) for the remaining six months of the experimental period which covered a total of one year. The tuberculin skin tests consisted of the intracutaneous injection of 0.1 ml. of a 5 per cent solution of a reliable commercial Old Tuberculin (OT) into epilated areas in the side. The administration of 0.1 ml. of a 10 per cent solution of the same material was frequently used in repeating doubtful reactions. These tests were read forty-eight hours after injection and were recorded in millimeters in diameter of induration. The relative size of the tuberculin reactions is represented by the symbols shown in tables 1 and 2.

Eleven of the animals in *Group 1* and 35 animals in *Group 2* were sacrificed at the end of a year and their tissues were studied grossly, histologically, and bacteriologically for evidence of tuberculous infection. The animals that died before the end of the experimental period were studied in the same manner as those that were sacrificed. The methods of study used were slight modifications of those used in the clinical laboratory to study human material.

Results

As shown in table 1, none of the animals of *Group 1* that received pyrazinamide either with isoniazid or with isoniazid and streptomycin developed a positive tuberculin skin test at any time. Autopsy examination on 6 animals of this group showed no evidence of tuberculous infection, either gross or microscopic. No tubercle bacilli were recovered by culture of the various organs of predilection.

[4] The Isoniazid (Nydrazid) used in this experiment was furnished through the courtesy of Dr. John T. Groel, Associate Medical Director, The Squibb Institute for Medical Research, New Brunswick, New Jersey.

[5] Pyrazinamide (Aldinamide) was furnished through the courtesy of Lederle Laboratories, Division of American Cyanamid Company, Pearl River, New York.

TABLE 1

THE EFFECT OF CHEMOTHERAPY UPON THE TUBERCULIN SKIN TEST IN GUINEA PIGS INOCULATED WITH THE H37Rv STRAIN OF TUBERCLE BACILLI AND THE AUTOPSY RESULTS

Treatment was Started at the Time of Inoculation

Guinea Pig No.	Days After Inoculation		Weeks After Inoculation															Autopsy Results		
	0	16	4	6	8	10	12	14	16	18	20	24	26	28	32	36	42	52	Gross	Histology	Bacteriology
	Isoniazid and Pyrazinamide													No Drugs							
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—Died, nontuberculous disease					—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Isoniazid and Streptomycin																				
6	—	—	●	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
7	—	—	●	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	●	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—
	Isoniazid and PAS																				
11	—	—	●	—	●	●	±	●	—	—	—	●	●	—	—	●	●	●	—	—	—
12	—	Died, nontuberculous disease																	—	—	+
13	—	Died, nontuberculous disease																	—	—	+
14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	■	⊕	+	+	+
15	—	Died, nontuberculous disease																	—	—	+
	Isoniazid and Streptomycin, and PAS																				
61	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•		
62	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•		
63	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•		
64	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•		
65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•		
66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•		
67	—	—	—	—	—	—	—	—	—	—	—	Died, nontuberculous disease							—	—	—

* These animals not sacrificed at this point.

—represents negative reaction; ± represents equivocal reaction. Other signs represent positive reactions as follows:

● = reaction of 8–10 mm. in diameter

■ = reaction of 15 mm. or more in diameter

● = reaction of 10–12 mm. in diameter

⊕ = reaction of 10 mm. or more in diameter with central necrosis

The animals of *Group 1* that received isoniazid-streptomycin developed slight evidence of skin sensitivity to tuberculin about a month after inoculation, but this quickly subsided and did not become manifest again during the remainder of the experiment. An interesting observation in this group was the recovery of living tubercle bacilli from the tracheobronchial lymph nodes and the spleen of animal *No. 6*, which had consistently failed to react to tuberculin for more

than ten months prior to autopsy. Grossly, the only evidence of tuberculous infection was one moderately enlarged tracheobronchial lymph node. In this animal the spleen appeared normal on gross examination. Microscopically, there were several very small sclerosing tubercles in the tracheobronchial lymph node and several cellular tubercles in the Malpighian bodies of the spleen. This animal showed no clinical evidence of tuberculosis. The other animals of this group were free from visible evidence of active tuberculosis at the time of autopsy.

Two surviving animals in the isoniazid-PAS group showed fundamentally different patterns of reaction to tuberculin and equally different patterns of response to the tuberculous infection. Animal *No. 11* had developed a weak tuberculin reaction one month after inoculation. The pattern of response fluctuated, ranging from a slight reaction to no reaction and back to a reaction again a number of times during a year of observation. There was no indication of active tuberculous infection at autopsy. Animal *No. 14* showed no evidence of tuberculin sensitivity until nine months after inoculation, which was approximately three months after chemotherapy had been discontinued. At this time a strong tuberculin reaction became manifest, and tubercle bacilli, which had apparently been dormant during this period, began to multiply, resulting in progressive clinical disease. Autopsy confirmed the presence of far advanced, active tuberculosis. The remaining animals treated with isoniazid-PAS died early of causes other than tuberculosis and will not be considered further.

Data are presented in table 2 on the animals in the delayed treatment group (*Group 2*). All animals in this group had developed weak to moderately severe skin sensitivity to tuberculin by the sixteenth day after inoculation, at which time multiple-drug therapy was started. Although the level of the tuberculin skin sensitivity fluctuated somewhat during the early weeks of chemotherapy, there was no definite indication of any animals becoming nonreactors to tuberculin until about two months later when the level of tuberculin skin sensitivity had decreased to a point of being questionable in 6 animals. At the twelve-week point in the six-month course of chemotherapy, 13 of the infected animals had become nonreactors, and after eighteen weeks of drug treatment all animals were nonreactors to tuberculin except 5 which showed only questionable response to tuberculin. After this point in the therapy program, tuberculin skin sensitivity appeared to increase slightly in some of the animals and fluctuated between negative and weakly positive for the remainder of the observation time. This pattern of response to tuberculin occurred to some extent in 20 of the 45 animals of this group. Because it began before chemotherapy was discontinued and continued after it was stopped, there was no definite correlation between drug therapy and the behavior of the tuberculin skin reaction in these animals after the initial two or three months of therapy.

The remaining 25 guinea pigs of *Group 2* became nonreactors after three to four months of chemotherapy and showed no further definite evidence of tuberculin skin sensitivity during the remainder of the year of observation, although 2 of the guinea pigs of this group (*Nos. 26* and *45*) yielded viable tubercle bacilli on culture at autopsy.

When chemotherapy was discontinued at the end of six months of treatment,

TABLE 2

THE EFFECT OF CHEMOTHERAPY UPON THE TUBERCULIN SKIN TEST IN GUINEA PIGS INOCULATED WITH THE H37Rv STRAIN OF TUBERCLE BACILLI AND THE AUTOPSY RESULTS

Treatment was Started Sixteen Days after Inoculation

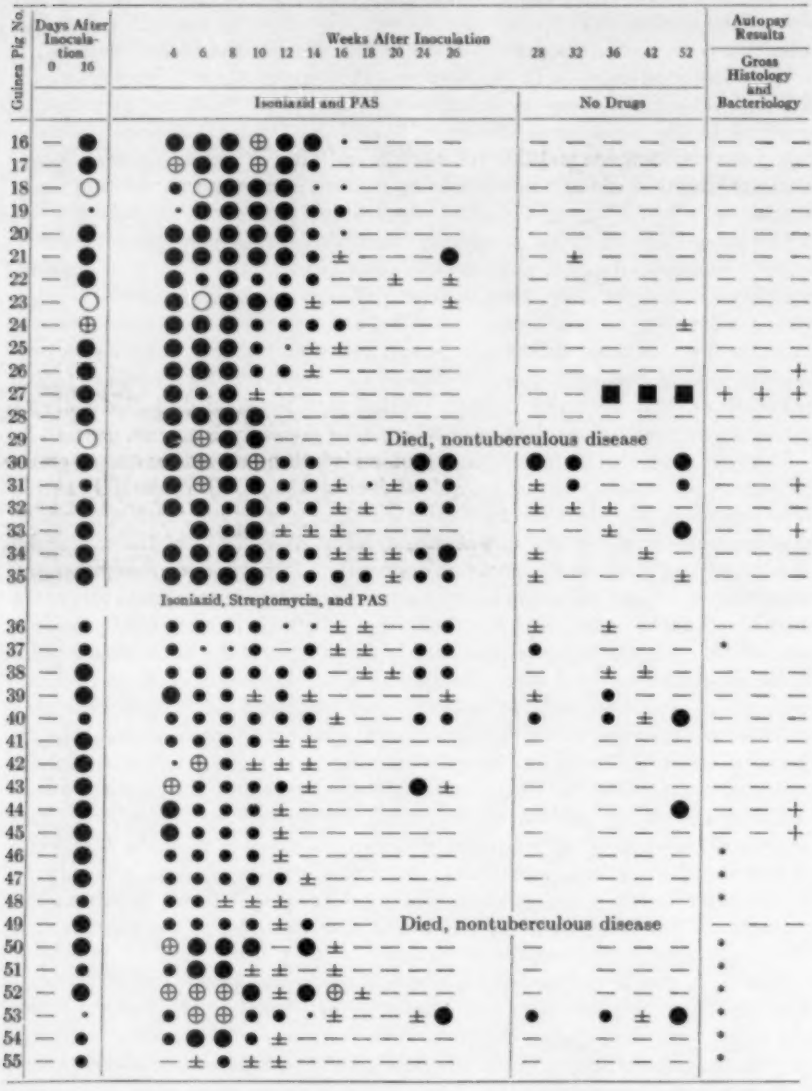

TABLE 2—CONCLUDED

Guinea Pig No.	Days After Inoculation 0	16	Weeks After Inoculation 4	6	8	10	12	14	16	18	20	24	26	28	32	36	42	52	Autopsy Results: Gross Histology and Bacteriology
			Isoniazid and PAS											No Drugs					
			Isoniazid and Streptomycin																
56	—	●	●	●	●	●	●	±	—	—	—	—	—	—	±	—	●	●	— — +
57	—	●	●	●	●	●	●	—	—	—	—	—	—	—	—	—	—	—	— — —
58	—	•	●	●	●	●	●	±	—	—	—	—	—	—	—	—	—	—	— — —
59	—	●	●	●	●	●	●	—	—	—	—	—	—	—	—	—	—	—	— — —
60	—	●	•	•	•	●	●	—	—	—	—	—	—	—	●	±	●	●	— — —

* These animals not sacrificed at this point.

— represents negative reaction; ± represents equivocal reaction. Other signs represent positive reactions as follows:

• = reaction of 5–8 mm. in diameter
● = reaction of 8–10 mm. in diameter
● = reaction of 10–12 mm. in diameter
■ = reaction of 15 mm. or more in diameter
⊕ = reaction of 10 or more in diameter with central necrosis
○ = reaction of 12–15 mm. in diameter

11 animals of *Group 2* were slightly sensitive to tuberculin and the 4 additional ones were questionably sensitive to this material. However, at the end of the six-month period of observation without chemotherapy, 7 of these 11 tuberculin skin-sensitive animals and the 4 additional questionable tuberculin-sensitive animals gave no tuberculin skin reactions. None of these animals showed any evidence of active tuberculous infection at the time of autopsy.

On the other hand, 5 animals with no reactions to tuberculin when chemotherapy was stopped later became reactors during the ensuing six months without chemotherapy. One of these animals (*No. 27*) had a marked tuberculin skin reaction and showed active, progressive tuberculosis at the time of autopsy. Four of these 5 guinea pigs had weak tuberculin skin reactions just before autopsy. Two of these yielded tubercle bacilli on culture from small foci at the subcutaneous inoculation site and one (*No. 33*) yielded tubercle bacilli from a slightly enlarged tracheobronchial lymph node. The remaining guinea pig (*No. 60*) had no evidence of active tuberculosis at the time of autopsy.

The 9 animals in which living tubercle bacilli were recovered at autopsy had had two or more negative tuberculin skin tests, some as many as fourteen over a period of ten months, between the time they were inoculated and the time autopsy was performed. This clearly indicated that there had been living tubercle bacilli in the animals' tissues when tuberculin skin sensitivity was below the point of being detectable by the methods used in this experiment. This represents about 20 per cent of the animals on which autopsy was performed.

Although tubercle bacilli were recovered from 7 animals that had had multiple-drug therapy for six months, beginning sixteen days after inoculation, only one (*No. 27*) showed evidence of progressive visceral tuberculosis. In this animal, a strong tuberculin skin reaction without variation had been present for several months just prior to autopsy.

Therefore, living tubercle bacilli were recovered from 9 of 48 animals sacrificed after receiving multiple-drug antimicrobial therapy for six months, followed by a six-month period of observation without drug therapy. Three of these animals had had negative tuberculin skin tests just prior to autopsy.

Discussion

In 1951 Mascher (8) reviewed some of the prevailing fallacies regarding the interpretation of the tuberculin skin reaction. He stated that there were only two major observations concerning the significance of a tuberculin reaction about which there seemed to be a unanimity of opinion. One concerns the view that a person who has never been infected with *M. tuberculosis* is absolutely insensitive to tuberculin even in large amounts. The other concept, the correctness of which is generally conceded, is that sensitivity to tuberculin in a person signifies that the subject tested harbors one or more foci brought about by infection with *M. tuberculosis*, either by natural infection or by vaccination. Mascher further concluded that a positive tuberculin reaction may spontaneously revert to negative not only in clinically healthy persons but occasionally in persons with active pulmonary tuberculosis. He also cited a series of eleven such cases, five of which were proved by guinea pig inoculation of the microorganisms recovered from the sputum of these patients.

Rich (9) also pointed out that clinically healthy persons may be nonreactors to as much as 10 mg. of OT and still harbor living virulent tubercle bacilli in old "healed" lesions. On the other hand, Pinner (10) called attention to the fact that the presence of viable tubercle bacilli is not a necessary condition for the persistence of a tuberculin skin reaction. These relatively infrequent exceptions to the generally accepted principles of interpretation of the tuberculin reaction do not invalidate its usefulness, but do serve to indicate that there are basic questions concerning this very useful and important test which are still unanswered.

There is no general agreement regarding the frequency of a spontaneous reversion to negative of previously positive tuberculin reactions in clinica'ly healthy subjects, nor is there complete agreement regarding the proper interpretation of such observations. Kellar and Kampmeier (11) found that 21.5 per cent of 130 medical students and nurses reverted during a period of four years. Dahlstrom (12) reported that 11.1 per cent of 2,490 reactors re-tested after five to fifteen years had reverted (1 mg.). Most other investigators have reached considerably lower figures for the incidence of spontaneous loss of cutaneous sensitivity to tuberculin. Lloyd and MacPherson (13) found 2 per cent reversion (1 mg.) in 200 children tested following the first test. Zacks and Sartwell (14) reported a 3 per cent incidence of reversion in more than 1,000 persons re-tested after ten years. Austrian (15), in a series of cases followed from eight to ten years, found that a sizeable number had reverted when re-tested by the Pirquet method but, when tested by the Mantoux method, none were insensitive to tuberculin. A careful analysis of all of these reports indicates that spontaneous reversion occurs most frequently in subjects who have very weak or questionable reactions initially

even to strong dilutions of tuberculin. Hardy (16) reported the highest percentage (6 per cent) of reversion in children. She also found that those children who had either normal chest roentgenograms or chest films showing only moderately enlarged tracheobronchial lymph nodes were most likely to revert to negative.[6]

The number of persons among those who cease being tuberculin reactors who also experience a bacteriologic cure of their infection is unknown. The difficulties of collecting such data are obvious. It is reasonable to assume that some of these people, particularly those with no reaction to strong dilutions of tuberculin for a period of years, had eradicated their infection.

Fluctuation in the level of tuberculin allergy in both man and animals has long been recognized. It is reasonable to believe that the allergy of the so-called "weak reactors" may at times fall below the level detectable with a given strength of tuberculin and later return to a level which would give a reaction. Tuxkey, DuFour, and Seibert (18) reported observing this phenomenon in student nurses who were tested at periodic intervals during their training. Willis (2) has observed marked fluctuation in the level of tuberculin skin sensitivity in guinea pigs. Practically all of the animals in the present study also exhibited to some extent this fluctuating type of tuberculin response while receiving antituberculous chemotherapy.

So many factors can influence the level of tuberculin skin sensitivity that an exact appraisal of the causes of the phenomenon become extremely difficult. Fundamentally, the ultimate development of tuberculous disease following infection with virulent tubercle bacilli depends upon whether the balance of causative factors favors the parasite or the host. It is conceivable that these factors may be so delicately balanced in some cases that the issue may shift back and forth between the host and parasite a number of times before finally being determined permanently in favor of one or the other. The waxing and waning of the tuberculin skin reaction as it occurred over a period of time in some of these animals may reflect this closely balanced relationship between the host and parasite. After the initial infection had been controlled by chemotherapy, this phenomenon seemed to occur in the animals irrespective of the continued use of antituberculous drugs.

Willis (2) has reported spontaneous loss of tuberculin skin sensitivity in guinea pigs infected with the attenuated strain R1Rv. Some animals in his series required two years or more to revert to negative. Vandiviere (19) has also reported that animals vaccinated with attenuated strains of varied potencies will vary in duration of allergy. Those vaccinated with certain strains of BCG may lose their allergy in a matter of six months while those vaccinated with certain other strains

[6] In a recent study of the "Prophylactic Effects of Isoniazid on Primary Tuberculosis in Children" by the U. S. Public Health Service, Ferebee and associates found that there was a slight reduction of the size of the cutaneous tuberculin reaction in two similar groups of children observed over a period of twelve months. One of these groups received isoniazid and the other group received a placebo. In these children, who had minimal tuberculous involvement, there was no evidence that tuberculin sensitivity decreases more frequently with isoniazid therapy than with placebo.

of BCG may last considerably longer. Animals vaccinated with R1Rv may maintain allergy for a period longer than two years.

In the present study, guinea pigs that received chemotherapy for tuberculous disease were observed to become insensitive to ordinary doses of OT, and about 20 per cent were later shown to harbor living tubercle bacilli. This is in agreement with the earlier reports mentioned at the beginning of this paper (5–7). It appears that the tuberculin skin test as performed in guinea pigs that received prolonged multiple-drug therapy for virulent tuberculous infection is not an absolute test for determining whether the tuberculous infection has been eradicated, even when the animals have been insensitive to tuberculin for a period of many months. On the other hand, a weak or questionable tuberculin skin reaction is not absolute proof that living tubercle bacilli exist in the tissues in sufficient numbers to be detected by the usual means of bacteriologic examinations.

In 7 of the animals that yielded tubercle bacilli at autopsy, the tuberculin skin test was negative for various lengths of time from thirty days to a maximum of nine months between the time of inoculation and death. There was no gross or histologic evidence of progressive tuberculous disease in these animals. Thus the animals harbored living tubercle bacilli which were producing little or no apparent injury. The factors responsible for these phenomena are complex and are not fully understood.

The long-term effects of isoniazid in rendering certain strains of tubercle bacilli avirulent for guinea pigs are well known. However, such bacilli are usually isoniazid-resistant and catalase-negative mutants. As the *in vitro* tests showed that the organisms that were eventually recovered in these current studies were isoniazid susceptible and catalase positive, drug resistance does not appear to be the logical explanation for their survival in the tissues.

Various aspects of these problems have been studied by many investigators. Canetti (20) presented a comprehensive discussion of the evolution of the pulmonary tuberculous lesion from the histobacteriologic point of view. He included observations made before the use of antituberculous chemotherapy and also observations upon lung specimens of persons treated by chemotherapy for tuberculous disease. This masterful review serves to emphasize the lack of complete understanding of many of the problems concerning the pathogenesis of tuberculosis. Dubos (21) pointed up the need for new techniques for studying these host-parasite relationships in the evolution of the tuberculous process. Apparently this development will be necessary before many of the drug-related fundamental principles of the pathogenesis of this disease are satisfactorily elucidated.

The factors responsible for the relative inactivity of tubercle bacilli which remain alive in guinea pigs tissues with negative or weakly positive tuberculin skin tests are poorly understood. Nevertheless, the state of the bacilli may be a reversible phenomenon in some cases, as evidenced by the 2 animals which later changed abruptly from being tuberculin nonreactors to strong tuberculin reactors and developed typically active tuberculosis. It is altogether probable that this same pattern might have been observed in other animals if the post-

chemotherapy observation period had been longer. On the other hand, a longer period of chemotherapy initially may have reduced the number of animals that were found at autopsy to harbor living tubercle bacilli. The type of chemotherapy suggests further possibilities in the degree of effectiveness that may be possible in establishing and maintaining this relative inactivity of the infecting tubercle bacilli in the host. It has been shown by McCune and his associates (22) that pyrazinamide in combination with certain other antituberculous drugs shows a definite superiority over other antituberculous drug combinations in reducing the number of viable tubercle bacilli in murine tuberculous infections. The same trend was suggested by results obtained in the present study, but the small numbers available for comparison were insufficient for making conclusions.

In those animals which had no reaction or a weak tuberculin skin reaction just prior to autopsy and from the tissues from which living tubercle bacilli were recovered, the histologic evidence of tuberculosis was confined to very small foci. These foci were cellular in structure and appeared fairly well encapsulated with fibrous tissue except for small lesions at the site of inoculation. These latter lesions showed microscopic areas of caseation. Lesions in the spleens of these animals were also microscopic in size and were for the most part cellular and within the Malpighian bodies.

Many attempts have been made to correlate the intensity of the tuberculin reaction with the clinical course of tuberculosis in man, both with and without drug therapy. The evidence is conflicting and difficult to assay. The results of this study suggest that there may be some such correlation in guinea pigs of the intensity of tuberculin reaction and the degree of activity of the disease process. The intensity of the initial tuberculin reaction waned somewhat gradually as treatment progressed and remained absent or at a low level in all animals that showed no extensive disease at autopsy. Moreover, the 2 animals with a 4-plus tuberculin reaction exhibited very extensive active tuberculosis at autopsy. These findings are not surprising and correlate well with what would logically be expected in this experimental situation.

Summary

A study has been made of the effect of chemotherapy upon the tuberculin skin reaction in 67 guinea pigs inoculated with the H37Rv strain of tubercle bacilli. Multiple-drug chemotherapy was given for a period of six months, utilizing isoniazid, streptomycin, pyrazinamide, and PAS in various regimens, followed by a six-month period of observation without drugs.

Nine of 48 animals sacrificed at the end of a year yielded culturable tubercle bacilli, and each of these 9 animals had had negative tuberculin skin tests at some time during the course of the experiment; 3 of these had been nonreactors for a period of nine consecutive months. It is apparent that virulent tubercle bacilli can live for extended periods of time in guinea pigs previously treated with prolonged multiple-drug chemotherapy without maintaining tuberculin skin sensitivity and without producing progressive disease.

There was correlation of the intensity of the cutaneous tuberculin reaction

and the degree of tuberculous activity within the animal tissues, and frequently the tuberculin skin reaction seemed to reflect changes in the host-parasite relationship when it was not evident by other means.

Sumario

Los Efectos de la Quimioterapia sobre la Cutirreacción a la Tuberculina en los Cobayos Infectados con la Cepa H37Rv del Bacilo Tuberculoso

Versa este estudio sobre el efecto de la quimioterapia sobre la cutirreacción a la tuberculina en 67 cobayos inoculados con la cepa H37Rv del bacilo tuberculoso. Se administró durante un período de seis meses una quimioterapia combinada, utilizando isoniacida, estreptomicina, piracinamida y PAS en varias combinaciones, seguido esto de un período de seis meses de observación sin drogas.

Nueve de los 48 animales sacrificados al cabo de un año rindieron bacilos tuberculosos viables y cada uno de estos 9 animales había mostrado cutirreacciones negativas a la tuberculina en alguna ocasión durante el transcurso del experimento; 3 de éstos habían sido negativos durante un período de nueve meses consecutivos. Es evidente que los bacilos tuberculosos virulentos pueden vivir durante períodos largos de tiempo en cobayos tratados antes con la quimioterapia combinada prolongada sin mantener cutisensibilidad a la tuberculina y sin producir enfermedad evolutiva.

Hubo correlación de la reacción cutánea a la tuberculina y la intensidad de la actividad tuberculosa en los tejidos del animal y frecuentemente la cutirreacción a la tuberculina pareció reflejar alteraciones en la relación de huésped-parásito que no eran manifiestas en otra forma.

Resume

Les effets de la chimiothérapie sur la réaction cutanée a la tuberculine chez le cobaye inoculé avec la souche H37Rv du bacille tuberculeux

Une étude des effets de la chimiothérapie sur la réaction cutanée à la tuberculine a été effectuée chez 67 cobayes inoculés avec la souche H37Rv du bacille tuberculeux. On a pratiqué la chimiothérapie avec l'isoniazide, la streptomycine, la pyrazinamide et le PAS en associations diverses pendant une période de six mois qui fuit suivie d'une période sans médicaments pendant six mois d'observation. Des bacilles viables ont été décelés chez neuf des quarante-huit animaux sacrifiés à la fin d'une année. Chacun de ces neuf animaux avaient eu des tuberculine-tests cutanés négatifs à un moment quelconque au cours de l'expérimentation; trois d'entre eux avaient été "négatifs" pendant une période de neuf mois consécutifs. Il apparaît que des bacilles tuberculeux virulents peuvent subsister pendant très longtemps chez le cobaye ayant été préalablement soumis à la chimiothérapie combinée, sans maintenir l'allergie cutanée à la tuberculine et sans entraîner une tuberculose évolutive.

Il existait une corrélation entre l'intensité de la réaction cutanée à al tuberculine et le degré de l'activité de la tuberculose dans les tissus des animaux et la réaction cutanée à la tuberculine a souvent paru refléter l'état des rapports entre le parasite et l'hôte alors qu'il n'était pas décelé par d'autres moyens.

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HINCONSTARCH IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1]

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(Received for publication October 28, 1957)

INTRODUCTION

A preliminary account (1) of a pilot investigation of hinconstarch (a polymer from periodate oxidized potato starch by condensation with equimolar proportions of isoniazid and *p*-aminobenzalthiosemicarbazone (2)) described the results obtained in twelve cases of pulmonary tuberculosis treated for three months. The present report deals mainly with the results obtained in 52 patients with pulmonary tuberculosis due to isoniazid-susceptible organisms and treated for periods of 3 to 12 months. The findings in 10 patients who exhibited tubercle bacilli resistant to 1 to 25 γ of isoniazid or more per ml. at the start of treatment are also presented in brief.

PLAN OF INVESTIGATION

Observation of Patients

Before the start of treatment, detailed clinical, laboratory, and roentgenographic studies were made to establish a baseline for each patient. To enable assessment of the response to hinconstarch and to detect toxicity (as well as making the routine clinical examinations), tests and estimations were repeated at intervals as follows:

Fortnightly determinations of sputum microscopy and culture for tubercle bacilli. (Normally, at the start of treatment when bacilli were plentiful, direct drug-susceptibility tests were done with Löwenstein-Jensen medium containing 0, 0.2, 1.0, 5.0, and 25.0 γ of isoniazid per ml. With less than 15 colonies on either of the two control tubes of medium, an indirect drug-susceptibility test was done. Subsequent attempts at cultivation of bacilli from each patient with initially isoniazid-susceptible tubercle bacilli were made on three tubes of medium (two plain and one containing 0.2 γ of isoniazid per ml.), an indirect susceptibility test being done in cases in which there were fewer than 15 colonies on a control tube and in all cases in which there was growth in the 0.2 γ per ml. tube. Whenever the patient was sputum free, gastric lavage specimens or laryngeal swabs taken on three consecutive days were each implanted on three tubes of medium as above. In all instances in which the tubercle-bacilli were isoniazid resistant *ab initio*, direct susceptibility tests were continued so long as the bacilli were sufficiently numerous. Otherwise, three tubes of medium, as above, were used with subsequent indirect susceptibility tests.)

Monthly determinations (more frequently if clinically indicated) were made of serum protein; albumin-globulin ratio; Van den Bergh; thymol turbidity; alkaline phosphatase, and blood urea; hematologic examinations (hemoglobin estimations, erythrocyte, leukocyte, and differential leukocyte counts, et cetera); and body weight.

At three-month intervals, erythrocyte sedimentation rate (Westergren, one hour) and a roentgenogram of the chest (including planigrams when indicated) were obtained.

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[1] From the Laboratories of the Medical Research Council of Ireland, Dublin; the Royal National Hospital for Consumption in Ireland; Ballyowen Sanatorium; and the Meath Hospital, Dublin, Ireland.

Particularly in the early stages of the investigation, the majority of the examinations were carried out at more frequent intervals than specified above. As well as the routine investigations, such supplementary tests as serum mucoprotein[2] and serum "hydrolyzable hydrazine" estimations (3), bone marrow examinations, erythrocyte fragility estimations, blood sugar, and urea clearance estimations were performed at intervals.

All examinations made before treatment to establish the current status of the patient were repeated at the end of each three months of hinconstarch therapy.

Roentgenographic Assessment

In assessing the roentgenographic appearances, both the extent of infiltration and the degree of cavitation were taken into account. Infiltration was assessed in terms of the number of lung zones involved, i.e., a possible total of 6 for both lungs. Partial involvement of several zones was recorded in terms of the equivalent number of complete zones. The smallest cavity was graded *1*; a moderate-sized single cavity or two or three small cavities were graded *2*; single large or multiple small (e.g., honeycombing) were graded *3*, and multiple large cavities were graded *4*.

All of the roentgenographic changes, taking into account the changes in the degree of infiltration and cavitation and the changes in the character of the infiltration (hardening or softening) were summarized in terms of *marked*, *moderate*, or *slight improvement; no change;* or *slight*, *moderate*, or *marked deterioration*. The roentgenographic assessments in the present report are the agreed readings of the three clinicians.

Selection of Patients

As in the pilot investigation (1), such patients were accepted as would ordinarily be treated with the usual multiple-drug regimens made up of streptomycin, isoniazid, or para-aminosalicylic acid (PAS), provided they had such clinical, bacteriologic, and roentgenographic signs as would enable changes during treatment to be readily assessed. Fifty-four patients in all were admitted to the investigation: 15 from the Royal National Hospital for Consumption in Ireland (N) (2 patients, however, had less than 3 months of treatment), 29 from the Meath Hospital (M), and 10 from Ballyowen Sanatorium (B). Only one of the 54 patients with isoniazid-susceptible tubercle bacilli had had previous chemotherapy for tuberculosis.

Hinconstarch Dosage

The drug was administered orally, initially in gelatin capsules containing 100 mg. but later in 200 mg. capsules, and occasionally in tablets of 250 mg. Doses of hinconstarch ranged from 0.5 gm. daily at the start of the investigation to 4.0 gm. daily when it had been found to be well tolerated. During the later stages of treatment, the tendency was to increase the doses for those patients who had been on low doses originally.

Duration of Hinconstarch Therapy

Thirty of the 52 patients who were treated for 3 months or longer had completed 12 months of therapy at the time of this report. In 6 of the remaining 22 patients, treatment was being continued—3 having completed but 6 months of treatment and three others, 9 months. Five patients who underwent surgery (one after 3 months, 3 after 6 months, and one after 9 months) were regarded as not continuing in the trial irrespective of whether they continued on hinconstarch or not. Two patients were self-discharged from the hospital after 3 and 6 months, respectively. One patient, discharged after 6 months, was considered

[2] A note on the changes in serum mucoprotein level will be published separately.

not to require further hinconstarch therapy. In 5 instances, in which the clinicians concerned were not satisfied with the patients' progress, treatment was changed; in one instance after 3 months, and in four instances after 6 months. Because of nausea and vomiting or dyspepsia, treatment was discontinued in 3 subjects after 9 months.

A fifty-third patient (a 33-year-old, 105 pound woman) with infiltration *4* and cavitation *4*, who was commenced on 1.2 gm. of hinconstarch daily, died from a massive hemoptysis after twenty-four days of treatment. In view of the short period of treatment, she is not considered elsewhere in this report.

Procedure in the Various Hospitals

There were some differences in procedure in the three centers. In center *N*, collapse therapy (phrenic crush with pneumoperitoneum) was instituted in 6 patients at a mean time of 5.7 months after the start of hinconstarch. Apart from center *M*, patients were hospitalized throughout treatment and were on modified bed rest. In center *M*, where the patients became ambulant at an early stage, the mean duration in the hospital for 26 of the 29 patients was 5.7 months. (Two patients who were transferred to another hospital for surgery and one whose failure to respond was associated with low serum concentrations of "hydrolyzable hydrazine" are omitted from this calculation).

Status of Patients at Commencement

There were 4 diabetics in the series of 52 patients and one of these also had osteitis deformans and tuberculous laryngitis. Another patient had severe Graves disease. Two patients were psychotics and another was a high-grade mental defective.

Twenty-four females aged 14 to 75 years (mean, 27.7 years) and 28 males aged 14 to 57 years (mean, 31 years) completed at least three months of treatment. The mean age for the entire group was 29.5 years, and in each of the three centers was essentially the same (29.3, 29.5, and 29.7 years).

Initially the general clinical condition (nutrition and musculature) of 25 of the patients was described as *good*, of 16 as *fair*, and of 11 as *poor*.

The appetite of 39 patients was described as *good*, of 10 as *fair*, and of 3 as *poor*.

A summary of the roentgenographic appearances at the start of treatment may be seen in table 2. According to the 1950 classification of the National Tuberculosis Association, except for one patient, all had moderately advanced or far advanced pulmonary tuberculosis.

Results

Clinical

As the assessment of both general condition and appetite is so largely subjective, reliance was placed on the objective criteria of weight, erythrocyte sedimentation, and bacteriologic change, together with the agreed roentgenographic interpretations of the three clinicians. All of these essential criteria are summarized in tables 1 through 4 or are included in the text.

Eight patients were febrile at the start of treatment, with mean evening temperatures (mean of 7 days) of 99.6 to 102.6° F. All except one were apyrexial at

TABLE 1

CHANGES IN SEDIMENTATION RATES (WESTERGREN, 1 HOUR)

Erythrocyte Sedimentation Rate	Number of Patients		
	Start	3 Months	6 Months
mm.			
0–10	14	32	37
11–20	12	9	6
21–50	18	11	6
51–	8	0	
Data not available			3

the end of 3 months, temperatures finally settling in 5, 32, 12, 12, 4, 86, and 5 days, respectively.

A mean increase of 10 pounds in body weight (range, 1 to 22 pounds) had occurred by the end of the first three months in 42 of 52 patients; a mean increase of 11.2 pounds (range, 1 to 29 pounds) had occurred by the end of the second three months in 41 of 48 patients; a mean increase of 13.9 pounds (range, 1 to 36 pounds) was present after nine months in 27 of 33 patients; and in 18 of 24 patients a mean increase of 15.4 pounds (range, 5 to 36 pounds) was present after one year. In those few patients who lost weight, the mean loss never exceeded 6 pounds.

The changes in the erythrocyte sedimentation rates during the first 6 months of therapy are shown in table 1. At 9 months only 3 patients had rates of more than 20, and at 12 months only one patient had a rate of more than 20. This latter patient had a feverish cold at the time of the determinations and there was no other evidence (clinical, roentgenographic, or bacteriologic) to suggest any deterioration in his pulmonary condition.

Roentgenographic Results

The roentgenographic results are summarized in tables 2 and 3. It may be seen (table 2) that 34 of 52 patients (65.4 per cent) at three months, 39 of 49 (79.6 per cent) at 6 months, 35 of 37 (94.6 per cent) at 9 months and 29 of 30 patients (96.6 per cent) at 12 months showed moderate or marked roentgenographic improvement or had shown clearing of the abnormal shadows.

All cavities were closed in 26 of 47 patients (55.3 per cent) who had cavities initially (table 3). Five of the other 21 patients were admitted to surgery and 4 were being continued on treatment.

Bacteriologic Observations

Sputum "conversion" (at least 3 consecutive fortnightly cultures and smears negative for tubercle bacilli) were obtained in 44 of the 52 patients (84.6 per cent) with a mean "conversion" time of 14.7 weeks (range 2–46), although only 30 patients had completed 12 months of treatment at the time of this report. Of the 49 patients who completed 6 months' therapy, 37 (75.5 per cent) showed sputum "conversion" at that time.

TABLE 2

SUMMARY OF ROENTGENOGRAPHIC FINDINGS (COMPARISONS WITH INITIAL ROENTGENOGRAMS)

At Start of Treatment			After 3 Months				After 6 Months					After 9 Months				After 12 Months		
			Improvement				Improvement					Improvement				Improvement		
Cavitation	Degree of Infiltration	Number of Patients	Cleared or Marked	Moderate	Slight	No Change	Cleared or Marked	Moderate	Slight	No Change	Deterioration	Cleared or Marked	Moderate	Slight	No Change	Cleared or Marked	Moderate	Slight
0	1 2 3	1 2 2 — 5		4 of 5	1 of 5		2 of 4	1 of 4			1 of 4	3 of 3				3 of 3		
1	1 2 3 4	3 2 5 1 — 11	1 of 11*	4 of 11	3 of 11	2 of 11	1 of 11	6 of 11	3 of 11	1 of 11		4 of 7	3 of 7			3 of 5	2 of 5	
2	1 2 3 4 5	2 5 3 3 1 — 14	4 of 14	8 of 14	2 of 14		5 of 14	8 of 14	1 of 14			4 of 14	7 of 14	1 of 14		7 of 11	4 of 11	

3	2 3 4 5	5 7 3 1 — 16	1 of 16	9 of 16	3 of 16	3 of 16	6 of 15	6 of 15	2 of 15	1 of 15		6 of 10	3 of 10		1 of 10	7 of 8		1 of 8
4	3 4 5 6	1 2 2 1 — 6	1 of 6	2 of 6	2 of 6	1 of 6	3 of 5	1 of 5	1 of 5			3 of 3				3 of 3		
Grand total = 52			7 of 52 65.4%	27 of 52	11 of 52	6 of 52	17 of 49 79.6%	22 of 49	7 of 49	2 of 49	1 of 49	22 of 37 94.6%	13 of 37	1 of 37	1 of 37	23 of 30 96.6%	6 of 30	1 of 30

* Roentgenogram of one patient not available for first 3 months.

TABLE 3

CAVITY CLOSURE IN 47 PATIENTS (BASED ON TRI-MONTHLY ROENTGENOGRAMS)

Time of Closure in Months	Number of Patients with All Cavities Closed
3	17
6	5
9	2
12	2
	Total.....26 of 47 = 55.3%

During their period of treatment, ranging from 3 to 12 months, bacteriologic conversion was obtained in 13 of 21 patients in whom the cavities remained open, as well as in all cases in which cavity closure was achieved.

Changes in isoniazid susceptibility were noted in the tubercle bacilli from 13 patients at some time during treatment. Because of the importance of this finding, the results in these 13 patients are given in some detail in table 4. It will be seen that 6 of these patients (*Nos. 10, 29, 37, 38, 45, 52*) who excreted bacilli of increased isoniazid resistance showed eventual reversal of infectiousness, and that satisfactory progress, both clinical and roentgenographic, allowed treatment to continue in another patient (*No. 43*). Of the remaining 6 patients, 2 (*Nos. 47* and *50*) were rendered fit for surgery, and the other 4 subsequently received streptomycin-PAS therapy. Only 5 (*Nos. 14, 29, 45, 47, 51*) of the above 13 patients had drug intakes of 40 mg. per kg. or greater during their first 3 months of treatment and, as will have been seen from the preceding account, progress was satisfactory in patients *Nos. 29, 45,* and *47*. Unsatisfactory progress in one (*No. 51*) of the 2 remaining patients (*Nos. 14* and *51*) was associated with poor absorption.

Drug Toxicity

In only 3 patients was it necessary to discontinue hinconstarch during the 12-month period on account of side effects (nausea and vomiting). In all (including the above 3 patients), nausea, with or without vomiting, was experienced in 13 patients. With the exception of 2, these patients, at the first experience of these symptoms, had drug intakes ranging from 45 to 66 mg. per kg. daily. One of these 2 patients had a long-standing duodenal ulcer, and the other had a pneumoperitoneum. These findings would appear to put the tolerable dosage at 40 to 45 mg. per kg. daily, although, as symptoms did not usually arise until after the third month, higher doses might be feasible during the first trimester (44 mg. per kg. gives the convenient calculation of weight in pounds/50 as the daily dose in grams).

With the exception of the 3 cases in which treatment had to be abandoned, temporary cessation of treatment for a period of 3 to 10 days or slight reduction of the dose enabled treatment to be continued.

One patient discovered that by emptying the capsules onto her food she had no discomfort. Since then other patients on hinconstarch have reported similar experiences.

Evidence of a mild hemolytic process was revealed by the Van den Bergh test.

TABLE 4

CHANGES IN ISONIAZID SUSCEPTIBILITY OF THE ORGANISMS FROM THIRTEEN PATIENTS

Patient Code Number	Hinconstarch Intake in First Three Months	Isoniazid Susceptibility of Organisms and Initial Roentgenographic Grading and Subsequent Progress
	mg/kg /day	
1	29	Resistant to 25 γ per ml. at 30 weeks. Starting: infiltration 1, cavitation 0. Moderate roentgenographic improvement, with bacteriologic conversion at 2 weeks, during initial 3 months in hospital. Moderate deterioration (sputum reversion at 20 weeks) during second trimester of domiciliary treatment. Hinconstarch discontinued.
9	29	Resistant to 25 γ per ml. at 14 weeks. Starting: infiltration 2, caviation 1. Initial moderate roentgenographic improvement not sustained. Hinconstarch discontinued after 6 months.
10	36	Resistant to 25 γ per ml. at 12 weeks and again at 20 weeks. Converted at 32 weeks. Starting: infiltration 2, cavitation 1. Sustained moderate roentgenographic improvement (cavity closure at 3 months) throughout the 12 months.
14	43	Resistant to 25 γ per ml. at 22 weeks. Starting: infiltration 3, cavitation 1. Slight roentgenographic improvement (cavity closure at 3 months) during the 6 months of therapy. Hinconstarch then discontinued.
29	62	Resistant to 25 γ per ml. at 8 weeks. Converted at 46 weeks. Starting: infiltration 4, cavitation 2. Marked roentgenographic improvement sustained from third to twelfth month.
37	26	Resistant to 0.2 γ per ml. at 4 weeks and to 25 γ per ml. at 10 weeks. Again susceptible at 18 weeks with conversion at 28 weeks. Starting: infiltration 3, cavitation 3. Marked roentgenographic improvement (cavity closure at 6 months) sustained from sixth to twelfth month.
38	32	Resistant to 1 γ per ml. at 10 weeks, then fluctuating between resistance to 0.2 γ per ml. and 5 γ per ml. until bacteriologic conversion at 32 weeks. Starting: infiltration 3, cavitation 3. Moderate roentgenographic improvement during first 6 months with marked improvement (cavity closure at 9 months) at 9 and 12 months.
43	18	Resistant to 0.2 γ per ml. at 10 weeks, then occasional positive cultures varying from susceptibility to 0.2 γ per ml. to resistance to 25 γ per ml. Starting: infiltration 4, cavitation 3. Marked roentgenographic improvement (cavity closure at 6 months) from 6 to 12 months.
45	40	Resistant to 25 γ per ml. at 16 weeks; then a series of negatives; a single positive at 38 weeks resistant to 25 γ per ml.; then a further series of negatives. Starting: infiltration 4, cavitation 3. Sustained moderate roentgenographic improvement throughout.
47	45	Resistant to 25 γ per ml. at 14 weeks, then fluctuating between susceptibility to 0.2 γ per ml. and resistance to 25 γ per ml. Starting: infiltration 3, cavitation 4. Moderate roentgenographic improvement during the first two trimesters, then thoracoplasty.
50	19	Resistant to 0.2 γ per ml. at 14 weeks, to 5 γ per ml. at 18 weeks and to 25 γ per ml. at 20 weeks. Starting: infiltration 5, cavitation 4. Slight roentgenographic improvement at 3 and 6 months (complete clearing on one side), then pneumonectomy.
51	57	Partial resistance to 25 γ per ml. at 6 weeks and apparently complete resistance at 10 weeks. Starting: infiltration 5, cavitation 6. Slight roentgenographic improvement at 3 months. Unsatisfactory progress associated with low blood concentrations of hydrolyzable hydrazine in spite of apparently adequate intake.
52	26	Resistant to 0.2 γ per ml. at 10 weeks; thereafter fluctuating between resistance to 1.0 γ per ml. and 5 γ per ml. until conversion at 40 weeks. Starting: infiltration 6, cavitation 4. Marked roentgenographic improvement (cavity closure at 6 months) sustained from the sixth to twelfth month.

Six patients gave single serum bilirubin values of 1 to 1.25 mg. per 100 ml. at 12, 5, 6, 3, one, and 8 months, respectively. With the exception of one patient, who had a hemoglobin of 10 to 12.5 gm. per 100 ml. during treatment (commencing at 13.5 gm. per 100 ml.) and alteration of the myeloid-erythroid ratio (11:1 at 3 months, 5:1 at 4 months, and 10:1 at 5 months), none of these 6 patients had any other evidence of erythrocyte disturbance. Two others gave repeated levels of 1 to 1.5 and 1 to 2.5 mg. per 100 ml. bilirubin values from the first month onwards, and the latter patient also showed evidence of slightly increased erythrocyte fragility at 6 months; but this was temporary. Both of these patients, however, gave hemoglobin values of 14.5 to 17 gm. per 100 ml. throughout treatment and normal blood pictures. Two patients showed slight temporary depression of neutrophil leukocytes at 6 months and at 3 months, respectively. Apart from the above instances, no other disturbance of the hemopoietic system was observed.

During their course of treatment, 5 patients showed the following abnormal values for the various liver function tests. One patient gave an alkaline phosphatase value of 21 units at 10 months; one who had an alkaline phosphatase of 24 units initially had values of 46 to 57 from the third month onwards; one had values of 19 and 22 at 8 and 9 months, and one had values of 15 to 19 between 6 and 12 months. Another patient had a thymol turbidity of 6 units at 3 to 6 months. All other results of the various liver function tests in the above patients and in all other patients were within the limits of normal. In no case was jaundice observed.

Three patients showed disturbance of renal function in the shape of glycosuria during the 12-month period. This occurred in one patient from the sixth month and in another temporarily during the eleventh month. In both of these the glycosuria was mild. In one patient the glycosuria, which was marked, occurred towards the end of the 12-month period. At the completion of the period of treatment it was also found that urea clearance in this patient was 37 per cent of normal. In all of the patients with lowered renal thresholds for sugar, the dosage had been 60 mg. per kg. or greater at the time of glycosuria. After cessation of treatment, biochemical tests and other tests in all patients showed a return to normal values. No patient who received less than 45 mg. per kg. showed any toxic symptoms which could with certainty be attributed to the drug.

Minor urticarial rashes appeared in 2 of the 52 patients at some time during therapy; one month and 19 days, respectively, after the start of treatment. The first patient responded to an antihistaminic cream, and in the second the rash cleared when treatment was suspended for 10 days. In both cases it was possible to continue treatment without recurrence of the rashes.

A fifty-fourth patient not included in the tables developed a blotchy macular rash on her tenth day of treatment which did not respond to antihistaminics. She was not rechallenged with hinconstarch.

Pharmacology

How the hinconstarch macromolecule is cleaved after ingestion is not yet understood. Urinary studies have shown that both active components of hin-

constarch are absorbed and metabolized. The known urinary metabolites of isoniazid (acetyl isoniazid, free isoniazid, *iso*nicotinic acid, *iso*nicotinyl glycine) have all been identified in the urine from hinconstarch patients as well as di-*iso*nicotinyl hydrazine (4, 5). The thiosemicarbazone moiety appears in the urine oxidatively cyclized to 5-*p*-acetamidophenyl-2-amino-1:3:4-thiadiazole (4).

The blood concentration of hinconstarch metabolites was measured by a colorimetric method (A) developed for isoniazid (3). The method used also estimates thiosemicarbazones.

The colored compounds obtained from isoniazid and from *p*-acetamidobenzalthiosemicarbazone have similar absorption spectra in the region 430 to 500 μ, with λ_{max} 460 μ, but the color values at this wavelength are in the ratio 4:1, respectively. Other metabolites which may be present in the blood would probably have different color values. For convenience, therefore, the total chromogenic material in the serum was calculated as "isoniazid." The figures have no absolute significance, but indicate relative blood concentration of hinconstarch metabolites.

A small number of estimates of the serum content of materials containing the isonicotinyl radical was also made by the method (*B*) of Rubin and associates (11). A comparison of the results obtained by the two methods showed that the "isonicotinyl" material appeared to comprise approximately one half of the total estimated by the former method. Again, these figures were not absolute because the method was developed for the estimation of isoniazid and its *iso*propyl derivative, whereas the color value of unknown derivatives may not be the same.

Preliminary experiments with method *A* indicated that maximal blood concentrations were reached about two and one-half hours after a dose. A sample of the values obtained in patients on various dosages is shown in table 5. The patients had been taking the drug for at least a week, and it was assumed that a stable condition had been reached. As may be seen in table 6, with a constant intake of drug, blood concentrations are well maintained throughout the day. However, absorption patterns are highly individual, as is evidenced by the figures in this table.

It has been found, that when the blood concentrations are consistently less that 1.5 γ per ml. (calculated as "isoniazid" according to method *A*), the therapeutic results may not be good. It is therefore advisable to estimate the concentrations of drug in all patients at the end of the first week of therapy.

TABLE 5

BLOOD CONCENTRATIONS CALCULATED AS ISONIAZID (HINCONSTARCH GIVEN IN THREE EQUAL DOSES—MORNING, NOON, AND EVENING)

Each Dose	Number of Patients	Mean Concentration (γ/ml. of Serum)	
		2½ Hours after Morning Dose	Range
mg.			
800	10	2.3	1.2–3.3
1,000	10	2.7	0.7–5.0
1,200	4	2.9	2.0–4.2
		Before morning dose	
1,000	8	2.7	1.0–5.0

TABLE 6

DIURNAL VARIATIONS IN "ISONIAZID" BLOOD CONCENTRATIONS (γ/ML.) IN 7 SELECTED PATIENTS RECEIVING HINCONSTARCH

Time	Patients and Their Total Daily Dose of Hinconstarch						
	No. 43 1.2 gm.	*No. 23* 2.4 gm.	*No. 37* 2.4 gm.	*No. 44* 2.4 gm.	*No. 33* 3.0 gm.	*No. 19* 3.0 gm.	*No. 29* 4.0 gm.
	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.	γ/ml.
Fasting	1.1	1.9	0.7	1.4	3.1	4.0	2.6
hours							
0	(0.4 gm. dose)	(0.8 gm. dose)	(0.8 gm. dose)	(0.8 gm. dose)	(1.0 gm. dose)	(1.0 gm. dose)	(1.0 gm. dose)
1¾					3.0	3.6	1.9
2	1.4	1.5	1.1	1.4			
3					(1.0 gm. dose)	(1.0 gm. dose)	(1.0 gm. dose)
4	(0.4 gm. dose)	(0.8 gm. dose)	(0.8 gm. dose)	(0.8 gm. dose)			
4½					4.9	4.2	2.4
5½	1.4	1.2	1.2	0.9			
6					(1.0 gm. dose)	(1.0 gm. dose)	(1.0 gm. dose)
					4.8	4.1	2.1
7	1.5	1.2	1.4	1.5			
8	(0.4 gm. dose)	(0.8 gm. dose)	(0.8 gm. dose)	(0.8 gm. dose)	5.1	6.6	2.3
9							(1.0 gm. dose)
10	2.1	1.7	2.4	1.9	5.0	8.4	2.3
12	2.4	1.4	2.1	1.3	3.9	6.6	2.5

Observations on 10 Patients Excreting Tubercle Bacilli Resistant to Isoniazid at the Start of Treatment

Apart from one patient with tubercle bacilli resistant to 1 γ of isoniazid per ml., all of the 10 patients excreting isoniazid-resistant bacilli (resistant to 5 to 25 γ or greater per ml.) had had previous and prolonged medication with isoniazid in combination with streptomycin and/or PAS without success.

Nine of these patients were females with a mean age of 28.7 years (range 16 to 50 years). The sole male was 41 years old.

The general condition of only 2 patients in this series was described as *good*; that of others was described as *fair*; and of 6 others as being *poor*. Appetite was *good* in 4, *fair* in 3, and *poor* in 3.

Six of the patients had *grade 4* cavitation with infiltrations ranging from 3 to 6. The other 4 patients had cavitation *2* or *3* with infiltrations of 1 to 6.

Two of the patients were febrile at the commencement of hinconstarch therapy. After 3 months, one was still febrile, and the temperature was normal in the other. However, one other patient became febrile during this period. Only 2 patients showed an increase in weight during the first three months of

hinconstarch therapy (2 pounds). The other 7 patients lost weight (2 to 6 pounds).

One patient showed a normal erythrocyte sedimentation rate throughout treatment. The rates of 3 patients showed a definite fall during treatment, but those of the remaining 6 patients were substantially unchanged.

One patient showed moderate roentgenographic improvement during the first 3 months which continued during the 9-month period of observation. Slight improvement was recorded in another patient, but the condition of 7 of the remaining 8 was unchanged roentgenographically and that of the other showed slight deterioration. Cavity closure was not attained in any of the 10 patients. One patient died after 4½ months.

Disappearance of tubercle bacilli from the sputum occurred in 2 patients, one with organisms initially resistant to 1 γ of isoniazid per ml. (not previously treated with isoniazid) and the other with bacilli resistant to 25 γ per ml., after 24 and 16 weeks, respectively.

The dosage in the isoniazid-resistant series was higher than in the isoniazid-susceptible series and 7 of 10 patients had intakes of greater than 45 mg. per kg. during their first 3 months of treatment (ranging from 47 to 82, with a mean of 60 mg. per kg.) as compared with 14 of 52 in the isoniazid-susceptible series. As might be expected, the incidence of side effects was greater, and in 5 patients it was necessary to discontinue treatment: in 3 on account of nausea, with or without thirst and vomiting; in one (the patient receiving the highest dose of the 64 patients) because of peripheral neuritis; and in one due to skin sensitivity. Occasional nausea and vomiting was also experienced in another patient, but it was not of sufficient severity to interrupt treatment. Because of their previous failure to respond to all other forms of chemotherapy, their isoniazid resistance, and their apparent lack of response to hinconstarch, it was not considered worth while to administer lower doses to these 5 patients.

Evidence of some slight hemolysis was present in 3 patients, serum bilirubin figures of 0.5–1.0 mg. per 100 ml. being obtained. Slight renal glycosuria was found in one patient during her last month of therapy.

Discussion

The present investigation has established that a dose of 40 to 45 mg. of hinconstarch per kg. daily gives an adequate therapeutic effect without toxicity. In arriving at this figure, doses of varying magnitude were employed, some too low, leading to slow response and isoniazid resistance; others too high, leading to toxicity. Yet, in spite of the exploratory nature of the investigation with regard to dosage, moderate or marked roentgenographic improvement was obtained in 65 per cent of patients at three months and in 79 per cent at six months, with greater percentages at later periods. Disappearance of tubercle bacilli from the sputum occurred in 84 per cent of patients.

These results compare favorably with the results of other published investigations using combinations of isoniazid, streptomycin, and PAS. For example, in the British Medical Research Council investigations (6) it was concluded, on

the basis of results at three months, that streptomycin (1 gm. daily) together with isoniazid (200 mg. daily) was the most effective drug combination studied. On this regimen, in 53 per cent of all patients, marked or moderate roentgenographic improvement was observed. In the British Medical Research Council studies, 19 per cent of the patients with cavitary disease were cavity free at the start of therapy as compared with 10 per cent in the hinconstarch group. The weight gain in the hinconstarch group averaged 7.7 pounds as against 12.4 pounds in the Medical Research Council streptomycin-isoniazid group; 7 of 8 patients who were febrile at the start were afebrile at 3 months on hinconstarch as against 75 per cent in the British group; and 42 per cent of those with sedimentation rates of 21 mm. or more showed a fall to 10 mm. or less, compared with 35 per cent in the Medical Research Council group. Tubercle bacilli were demonstrated in all patients in both investigations at the start. Single examinations (smear and culture) at 3 months gave 56 per cent negative results in the hinconstarch group as against 68 per cent in the Medical Research Council group. However, comparisons are not possible with the British group for longer periods of treatment. At 6 months, however, the 75.5 per cent "conversion" rate in the hinconstarch group compares favorably with the 64 to 78 per cent conversion rate obtained with various combinations of isoniazid, PAS, and streptomycin in the U. S. Veterans Administration studies in patients observed for five to eight months (7).

The hinconstarch results (65 per cent moderate or marked roentgenographic improvement at 3 months) also compare favorably with the 48 per cent obtained in the United States Public Health Service Cooperative Investigation (8), in which the high dose of 10 mg. of isoniazid per kg. daily was given in combination with 12 gm. of PAS daily and 1 gm. of streptomycin twice weekly.

That the high percentage of substantial roentgenographic improvement observed with hinconstarch is not due to bias in the interpretation of the roentgenograms is indicated by the closure of all cavities in 26 of the 47 patients (55 per cent) who had them initially, although only 27 of these 47 patients had completed 12 months of therapy with hinconstarch. This rate may be compared with the 37 to 54 per cent cavity closure rate obtained in the Veterans Administration studies in patients without surgery, or even with the 50 to 60 per cent closure rate in patients with or without surgery and observed for 9 to 12 months (7).

In synthesizing hinconstarch the aim was to produce a single acceptable compound for oral administration which would utilize the high antituberculous activity of isoniazid and which, at the same time, would be slow to permit the development of isoniazid resistance. With the recommended dosage of hinconstarch, the nausea which frequently accompanies oral administration of PAS is avoided, as is the necessity for intramuscular injection required by streptomycin. The evidence also suggests that, with adequate dosage, the emergence of resistant organisms will be no greater a problem than it is with any multiple-drug therapy in present use.

The obligation to use isoniazid in multiple-drug regimens so as to delay isoniazid resistance and thus prolong the duration of activity has led to a series

of clinical dilemmas. The toxicity of pyrazinamide and of the thiosemicarbazones has precluded their widespread use as adjuvants to isoniazid although it has been established experimentally that the thiosemicarbazones are most suitable adjuvants to isoniazid from a bacteriologic viewpoint (9). The necessity to use isoniazid in conjunction with PAS or streptomycin means, however, that if resistance develops—as it will do in a proportion of cases—there will be an inadequate response to combined streptomycin-PAS.

In a recent report, Katz and associates (10) stated that hinconstarch is effective in the treatment of acute severe pulmonary tuberculosis. Sputum "conversion" was striking. The symptomatic and roentgenographic improvements were as satisfactory as those obtained with currently used regimens. These writers also suggested that roentgenographic changes occurred more readily with hinconstarch.

Hinconstarch at a dosage level of 40 to 45 mg. per kg. would appear therefore to offer an attractive treatment for pulmonary tuberculosis. If it were employed as a first treatment, it would leave other combinations such as streptomycin and PAS as effective alternatives should the occasion for their use arise.

Summary

Fifty-two patients with pulmonary tuberculosis due to isoniazid-susceptible tubercle bacilli have been treated with hinconstarch for three to twelve months. Moderate or marked roentgenographic improvement was observed in 65 per cent of patients at three months and in 80 per cent at six months, with increasing percentages at longer intervals. Closure of all cavities was achieved without surgery in 55 per cent of patients during the period of the trial. Disappearance of tubercle bacilli from the sputum occurred in 85 per cent of the 52 patients (75.5 per cent at six months).

A daily dosage of 40 to 45 mg. per kg. is an effective dose of hinconstarch which does not cause toxic symptoms. Doses substantially larger than this may result in gastrointestinal or renal toxicity.

In 2 of 10 patients (9 of whom had had extensive chemotherapy previously) whose bacilli showed a varying degree of isoniazid resistance, bacteriologic "conversion" was observed.

Sumario

El "Hinconstarch" in el Tratamiento de la Tuberculosis Pulmonar

A 52 enfermos de tuberculosis pulmonar debida a bacilos tuberculosos isoniacido-susceptibles se les trató con "hinconstarch" durante tres a doce meses. Se observó mejoría radiográfica moderada o notable en 65 por ciento de los enfermos a los tres meses y en 80 por ciento a los seis meses, con porcentajes mayores a plazos más largos. Se obtuvo clausura de todas las cavernas sin cirugía en 55 por ciento de los sujetos durante el período de la prueba. Ocurrió desaparición de los bacilos tuberculosos del esputo en 85 por ciento de los 52 enfermos (75.5 por ciento a los seis meses).

Una posología diaria de 40 a 45 mg. por kg. constituye una dosis eficaz de hinconstarch, que no provoca síntomas tóxicos. Dosis substancialmente mayores que éstas pueden dar por resultado intoxicación gastrointestinal o renal.

En 2 de 10 enfermos (9 de los cuales habían recibido antes quimioterapia en gran escala),

cuyos bacilos revelaban en grado variable resistencia a la isoniacida, se observó "viraje" bacteriológico.

RESUME

L'Hinconstarch dans le traitement de la tuberculose pulmonaire

Cinquante-deux malades atteints d'une tuberculose pulmonaire dûe à des bacilles tuberculeux isoniazido-sensibles ont été traités par l'hinconstarch pendant trois à douze mois. Une amélioration modérée ou marquée de l'aspect radiologique a été observée chez 65% des malades au bout de trois mois et chez 80% au bout de six mois, cette proportion s'accroissant à mesure que l'intervalle était plus long. L'oblitération de toutes les cavernes a eté réalisée sans intervention chirurgicale chez 55% des malades durante la période de l'expérimentation. La disparition des bacilles tuberculeux dans l'expectoration s'est manifestée chez 85% de ces 52 patients (75,5% au bout de six mois).

L'hinconstarch à la dose quotidienne dex 40 à 45 mg/kg est efficace et n'entraîne pas l'apparition de symptômes toxiques. Des doses nettement plus élevées que celles-ci peuvent présenter une toxicité gastro-intestinale ou rénale.

Chez 2 des 10 malades (9 d'entre eux ayant été soumis antérieurement à une chimiothérapie extensive) dont les bacilles présentaient divers degrés de résistance à l'isoniazide, la négativation de l'expectoration a été observée.

Acknowledgments

The assistance of Dr. P. Browne and Dr. D. Costello in this investigation is gratefully acknowledged. The writers are indebted to the Lasdon Foundation, Inc., New York, and to Messrs. Arthur Guinness, Son and Co., Ltd. for financial aid. The hinconstarch was supplied for this investigation by the Nepera Chemical Co., Yonkers, New York, to whom we are grateful. Thanks are due to Mr. D. Malone and Miss B. O'Connor for technical assistance. We are obliged to Dr. L. K. Malley for permission to include one of his cases in this report.

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BEHAVIOR OF THE "ATYPICAL" MYCOBACTERIA IN HELA CELLS[1,2]

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(Received for publication November 29, 1957)

The term "atypical" as applied to mycobacteria has been used to designate the nontuberculous acid-fast bacteria obtained from human specimens. It is now known that there are two groups of mycobacteria in this category, the "yellow" bacilli (1) and the "Battey" type (2), that are associated with pulmonary disease that closely resembles tuberculosis, both clinically and pathologically. The "yellow" bacilli form yellowish pigment when stimulated by light, i.e., they are photochromogenic, whereas the "Battey" type show little pigment when grown in the light or dark (3).

HeLa cells have been found to provide favorable sites for the growth of tubercle bacilli and several other mycobacteria capable of causing disease in humans (4–6). Mammalian tubercle bacilli of original pathogenicity grow in the cytoplasm of HeLa cells with specific cording not seen with other mycobacteria, whereas the strains of tubercle bacilli of reduced pathogenicity, such as BCG and H37Ra, grow much more slowly and in characteristic patterns. Among the mycobacterial species causing human disease, *M. balnei* and *M. fortuitum* each grow in these cells in their own pattern. *M. ulcerans* has so far been observed to grow only in monkey kidney tissue cultures (6). The explanation for its failure to grow in HeLa cells may lie in the characteristic slowness of its growth.

The behavior of the "atypicals" when introduced into HeLa cells is described in the present paper. Although the work with the "Battey" type of organisms has not reached a satisfactory stage of completion, the results to date with both groups of organisms are being reported because of the obvious importance that these widespread human pathogens become classifiable. The term "atypical" gives rise to considerable imprecision. It has been used for any of the poorly defined mycobacteria of questionable etiologic significance that are occasionally found in sputum specimens.

Materials and Methods

The methods used are described in detail elsewhere (4, 6). The mycobacteria are introduced into HeLa cells growing on coverslips, and the intracellular growth of the mycobacteria is observed by microscopic examination of coverslips removed at appropriate intervals and stained by acid-fast procedures. Mycobacterial cultures were maintained on Löwenstein-Jensen medium. Suspensions of mycobacteria for inoculation into tissue cultures are grown in Tween®-albumin medium for about six days, then centrifuged, and resuspended in Hanks' balanced salt solution (BSS). Just before the mycobacterial suspensions are added to the tissue culture tubes, the medium in them is changed to "infection medium," and the following day it is changed back to "growth medium." Since entry of

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[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

TABLE 1

THE CULTURES OF "ATYPICAL MYCOBACTERIA" THAT WERE STUDIED

	Strain Designation	Source
"Yellow" bacilli	*a*. Ba.	Dr. Ann Pollak
	b. No. 1	Sunmount package collection of Dr. Ernest Runyon; *Case 1* of Buhler and Pollak (1)
	c. No. 4	Sunmount package; Hall, Veterans Administration Hospital, Minneapolis (7)
	d. No. 8	Sunmount package; *Case 2* of Wolinsky *et al*. (8)
	e. Lo.	Mrs. Carolyn McDermott, Alabama State Health Department
	f. Fr.	Dr. J. K. Frenkel, University of Kansas Medical School: isolated from a hamster
"Battey" type of bacilli	*a*. No. 2	Sunmount package; from patient Albert W., Battey State Hospital, Georgia
	b. No. 3	Sunmount package; from patient Cordie P., Battey State Hospital, Georgia
	c. No. 7	Sunmount package; from patient Jesse G., National Jewish Hospital
	d. to *n*. Pa, Ch, Da8, Da7, Wo, St, Ki, Jo, Sm, Mc, In.	Mr. C. Edwin Smith, Battey State Hospital, Rome, Georgia. Similar to those described by Crow *et al*. (2)

mycobacteria into HeLa cells is very much greater in "infection medium" than in "growth medium," infection of the cells is largely confined to one day, and the subsequent developments of intracellular mycobacteria are reasonably uniform from cell to cell. HeLa cells in very good condition seem to be required for the development of useful growth patterns with both "yellow" bacilli and the "Battey" type of organisms. This may be related to the length of these mycobacteria, which requires that the HeLa cells be very well flattened out on the glass in order to provide adequate intracytoplasmic accommodation.

The cultures used and their sources are given in table 1. Their characteristics on bacteriologic media were identical with those described (1, 2).

RESULTS

"Yellow" bacilli: The six cultures of "yellow" bacilli formed a homogeneous group. All grew fairly rapidly in HeLa cells, and by the fifth day the picture was that shown in figure 1. The individual bacilli were long and their sides parallel. Regularly spaced along the entire length of most of the organisms were small beads, which were separated by intervals approximately equal to the width of the bacilli, and gave each bacillus the appearance of a ladder. Although beading has been seen in the other mycobacterial species studied, it has not been so well developed and uniform as it is with the "yellow" bacilli, and recognition of cultures of this mycobacterium has not been difficult. A similar beading is irregularly seen in the inocula of this organism prepared from growth in bacteriologic medium.

Five of the six cultures of "yellow" bacilli originated from human patients.

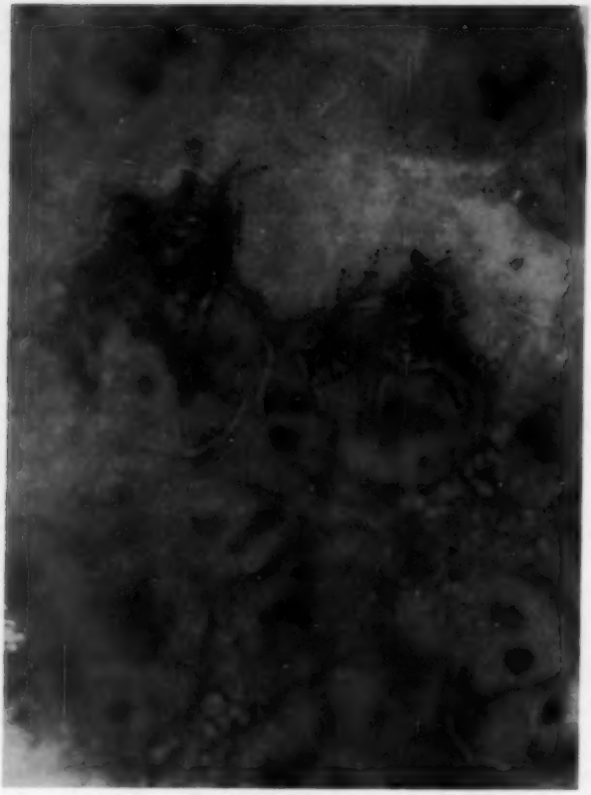

FIG. 1. "Yellow" bacilli in HeLa cells. Growth in five days. There is characteristically spaced beading along the entire length of nearly every organism. Strain Ba. (880 ×)

The other one, which was received from Dr. Frenkel, was isolated from a hamster that had been receiving injections of radioactive iodine and was not exposed to animals that had been inoculated with clinical material or cultures arising therefrom. The conditions suggested that the source of infection was the solution of radioactive iodine, which was kept at room temperature without sterile precautions (9). It would thus seem possible that study of the growth pattern developed in HeLa cells could be helpful in recognizing isolates from sources other than human patients. The rarity with which secondary cases are encountered in the families of patients infected with these organisms would suggest that nonhuman sources should be considered.

"Batley" type: The cultures of this group have not behaved in a homogeneous fashion, and a variation in morphology is observed. The most helpful diagnostic feature is long, branching filaments (figures 2 and 3). Bacillary growth with a

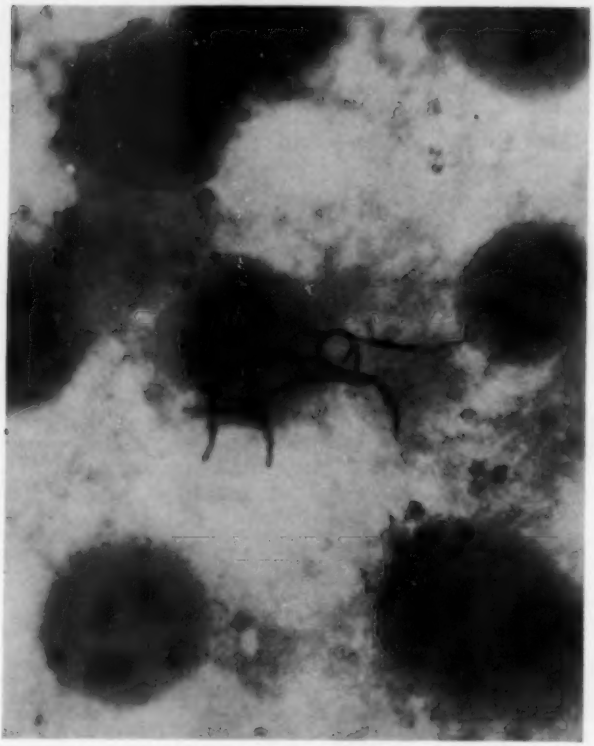

FIG. 2. "Battey" type. Three days after inoculation into HeLa cells. Branching is distinct and gives rise to a characteristic arrangement of the bacilli. Strain No. 3. (880 ×)

slight tendency to parallel alignment is common (figure 4), and coccal forms are also seen frequently with some cultures. The branching filaments are frequent with certain strains, e.g., Wo and No. 7, and these strains have shown only infrequent coccal forms. Others, such as Da 8, show many coccal forms and few branching filaments. The impression is gained that the intracellular growth proceeds from branching filaments and bacilli, but not from the cocci. Intracellular growth is usually accompanied by a marked decrease in the average length of the organisms.

Although exact comparisons are difficult because of the great differences in morphology, it is apparent that the "Battey" type grow in the cells at about the same rate as "yellow" bacilli, and both of these grow more rapidly than mammalian tubercle bacilli and more slowly than *M. fortuitum* or *M. marinum* and *M. balnei*.

The relative frequency of branching filaments, or coccal forms, seen with a given strain has been stable over a period of one year, with intermediate transfers

FIG. 3. "Battey" type. Two days after inoculation into HeLa cells. The long, branched filaments may be seen in this figure also. Strain No. 7. (880×)

every three months. The characteristic of the strain to form filaments or coccal forms can also be made out in smears of the inocula, which are prepared from growth in liquid medium or in smears made directly from growth on slants of Löwenstein-Jensen medium. However, the morphology, especially of the branched filaments, is seen with much more clarity in the HeLa cell preparation. Filtration of a suspension through filter paper tends to remove branched filaments selectively, and it is advantageous to omit this step in the preparation of the inocula for tissue cultures when studying these organisms.

Definite and frequent branching has also been observed in HeLa cells with a strain of *M. fortuitum* (5). The much more rapid growth of *M. fortuitum*, however, allows its differentiation from the "Battey" type.

The frequent branching seen with the "Battey" type of organisms emphasizes the perplexing problems of classification that arise in the area that lies between mycobacteria and nocardia. A culture, labeled *Nocardia intracellularis* (10), recently received through the courtesy of Dr. N. F. Conant of Duke University

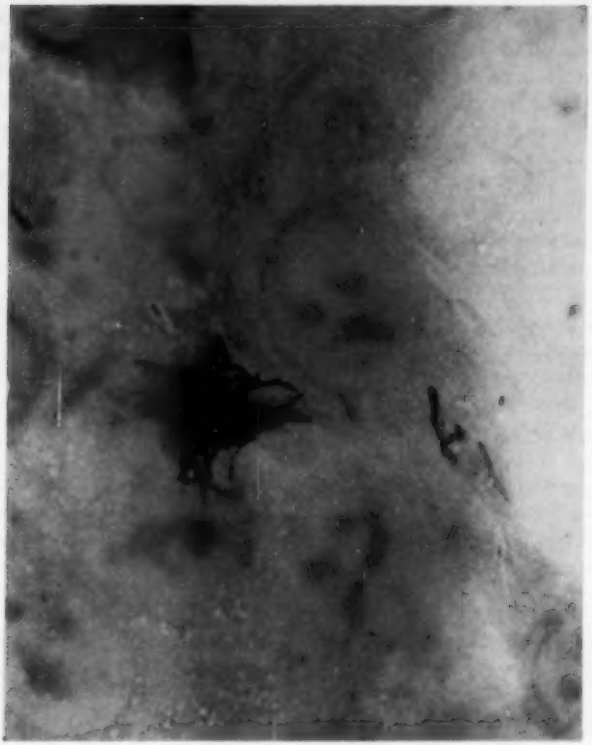

FIG. 4. "Battey" type. Two days after inoculation into HeLa cells. There is some parallel alignment but the growth pattern here is not especially characteristic. Strain No. 2. (880 ×)

School of Medicine, could not be distinguished from the frequently branching "Battey" types, such as Wo, either by its growth pattern in HeLa cells or its growth on Löwenstein-Jensen or Saboraud, or in Tween-albumin medium. Another strain, marked *Nocardia intracellularis*, received from the Veterans Administration Hospital in Minneapolis was not the same. Its growth on Löwenstein-Jensen medium was dry and could not be suspended in saline solution, whereas the Duke culture and all of the "Battey" type of cultures had moist growth that was easily suspended in saline. The growth of the Minneapolis culture on Saboraud medium was much greater than that of the Duke culture or the "Battey" types.

DISCUSSION

A great deal of help was provided by the Sunmount collection of "unknowns." The identification of the human and bovine strains of *M. tuberculosis* was made with considerable confidence. The growth pattern of the "yellow" bacilli was

also easily recognized. Cultures of the "Battey" type were in each case identified by the detection of branched filaments, but the growth patterns varied considerably between strains, and their correct labeling was difficult. The growth patterns formed in HeLa cells by both "yellow" bacilli and the "Battey" type of organisms were distinct from those developed by the other human pathogenic mycobacteria so far studied, which are *M. fortuitum*, *M. marinum* (and *M. balnei*), *M. ulcerans*, and *M. tuberculosis* (4–6). *M. leprae* has also been studied, but its growth in these cells has not been apparent (6).

The results reported here serve to illustrate how more information may be gained when an infectious agent grows in the host than when it characteristically fails to grow. For example, the failure of the "atypicals" to produce progressive disease in guinea pigs serves to differentiate them from pathogenic strains of tubercle bacilli, but does not allow them to be distinguished from the numerous other mycobacteria and other infectious agents that do not produce progressive disease in these animals. On the other hand, the growth of the "atypicals" in HeLa cells allows them to exhibit their specificity, so that it may be seen that they behave in characteristic fashion, which, at least in the case of the "yellow" bacilli, suggests distinct species. No difficulty has been encountered in differentiating "yellow" bacilli or the "Battey" type of organisms from tubercle bacilli which had lost pathogenicity for laboratory animals or man, or from isoniazid-resistant strains of tubercle bacilli.

Summary

The growth of "atypical" acid-fast bacilli in HeLa cells was studied according to the techniques previously developed for the study of other pathogenic mycobacteria.

The "yellow" (photochromogenic) bacilli grew intracellularly in characteristic patterns, the most prominent feature of which was a distinct beading which occupied the entire length of the organism. All cultures behaved uniformly, including one isolated from an infected hamster.

The "Battey" type of organism also grew in HeLa cells. Although much of the intracellular growth was not highly specific in pattern, branching filaments could always be found, and with some strains they were frequent.

The growth rates of both of these acid-fast bacilli in HeLa cells were intermediate between mammalian tubercle bacilli and *M. marinum* and *M. fortuitum*.

Sumario

El Comportamiento de las Micobacterias "Atípicas" en las Células HeLa

Se estudió la proliferación de los bacilos ácidorresistentes "atípicos de acuerdo con las técnicas elaboradas antes para el estudio de otras micobacterias patógenas.

Los bacilos "amarillos" (fotocromógenos) crecieron intracelularmente en patrones típicos, cuya característica más destacada consistió en granulación bien definida que ocupaba todo el largo del microbio. Todos los cultivos se comportaron del mismo modo, comprendiendo uno aislado de un microto infectado.

Los microbios del tipo "Battey" también formaron colonias en las células HeLa. Aunque una gran parte de las colonias intracelulares no fué muy específica en su patrón, podían encontrarse siempre filamentos ramificantes, siendo éstos frecuentes en algunas cepas.

Las tasas de proliferación de ambas variedades de estos bacilos ácidorresistentes ocupaban un puesto intermedio entre las de los bacilos tuberculosos de mamífero y el *M. murinum* y el *M. fortuitum*.

Resume

Comportement des mycobactéries "atypiques" dans les cellules HeLa

La croissance des bacilles acido-résistants "atypiques" dans les cellules HeLa a été étudiée conformément aux techniques précédemment mises au point pour l'étude d'autres mycobactéries pathogènes.

Les bacilles "jaunes" (photochromogènes) se développaient dans la cellule selon des types caractéristiques, des granulations distinctes occupant toute la longueur du bacille représentent l'aspect le plus commun. Toutes les cultures se sont comportées uniformément, y compris celle qui avait été isolée chez un hamster infecté.

Le type "Battey" de mycobactérie se développait également dans les cellules HeLa. Bien que la croissance intra-cellulaire n'ait pas présenté un type très spécifique, des filaments arborescents étaient constamment observés; ils étaient trés fréquemment recontrés dans certaines souches.

La vitesse de la croissance de ces deux bacilles acido-résistants dans les cellules HeLa était intermédiaire entre celle des bacilles tuberculeux de mammifère et *M. marinum* et *M. fortuitum*.

Acknowledgment

The excellent technical assistance of Mrs. M. Nanett Jackman and Mrs. Mary E. Jones is gratefully acknowledged.

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STUDIES WITH STREPTOVARICIN IN THE TUBERCULOUS GUINEA PIG[1]

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(Received for publication October 31, 1957)

Streptovaricin[2] has been reported (1) to have good *in vitro* activity against both virulent and saprophytic mycobacteria. In addition, studies by Rhuland and associates (2) have indicated that streptovaricin is able to protect mice infected with *Mycobacterium tuberculosis* var. *hominis* (H37Rv) or *M. tuberculosis* var. *bovis* (Vallée).

The work reported here is an investigation of the efficacy of streptovaricin in the tuberculous guinea pig. A preliminary report of these data was presented at the Veterans Administration—Armed Forces Conference on the Chemotherapy of Tuberculosis, February, 1957 (3).

METHODS AND MATERIALS

Mycobacterium tuberculosis var. *hominis* (H37Rv) was maintained on Löwenstein agar slants in the manner previously described (2).

In the studies of infected animals, tuberculin-negative guinea pigs weighing 400 to 500 gm. were used. As described in the method of Karlson and Feldman (4), the animals were inoculated subcutaneously over the sternum with 0.5 ml. of a culture of *M. tuberculosis* (H37Rv) containing 4.5×10^4 viable units per ml. At 21 days post infection these animals were divided into nine groups of 4 guinea pigs each. Five of these groups received 100, 50, 25, 12.5, and 6.25 mg. of streptovaricin. The sixth group received 5 mg. of isoniazid, while the seventh group was administered 15 mg. of streptomycin sulfate. The eighth group received a lactose placebo, while the ninth group received no treatment. Streptovaricin and isoniazid were prepared in gelatin capsules using lactose as a bulking agent. Streptomycin was prepared as an aqueous solution and was injected by the intramuscular route. Treatment was initiated at 21 days post infection and was continued daily for 62 days.

At the termination of the study, surviving guinea pigs were killed and gross pathologic examinations were made. Portions of certain lymph nodes (axillary, superficial inguinal, and tracheobronchial), lungs, spleen, and liver from each guinea pig in the eight experimental groups were fixed in formalin, 1:10. Tissues from the site of inoculation in guinea pigs from the above groups and from the untreated group were not examined histologically. Sections of the fixed tissues were stained with Harris' hematoxylin and eosin and Kinyoun's stain for acid-fast bacilli.

The toxicity of streptovaricin was determined in guinea pigs in the following manner: Each of 4 male guinea pigs was given a capsule of 125 mg. of streptovaricin daily. Four other male guinea pigs received empty capsules and served as toxicity-control animals. This dosage of streptovaricin was comparable with the 100 mg. dose in the studies of infected animals described below. At the termination of the 28-day toxicity experiment, heart blood was collected in heparinized vials from all animals of both groups. Leukocyte counts, hematocrit and photoelectrometric (5) hemoglobin readings were made from these blood samples. Differential leukocyte counts (200 cells counted) were made from Wright-stained blood films. Tissues of these animals were processed as previously mentioned. In addition, selected tissues were stained with Oil Red O® for fat.

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[1] From the Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.

[2] The trade name of The Upjohn Company for the antimicrobial streptovaricin is Dalacin.

Experimental

The demonstration that streptovaricin was efficacious in mice infected with *M. tuberculosis* (H37Rv) indicated that the drug was capable of suppressing an acute fulminating infection. It was of interest, therefore, to determine the efficacy of this drug in a chronic tuberculous infection in the guinea pig.

Toxicity studies in guinea pigs: Subacute toxicity determinations in guinea pigs revealed that, in the drug group, the average weight gain was 7.5 per cent, whereas the control group animals gained an average of 22.5 per cent. The importance of this difference was partially offset by the variation in starting weights of the 4 animals in each group. Likewise, the interpretation of the significance of somewhat larger adrenal gland weights in the group of streptovaricin-treated animals (0.075 per cent of body weight as compared with 0.60 per cent in the control group) was subject to the body weight inequality of the two groups.

At necropsy, mild to moderate splenomegaly was found in the 4 streptovaricin-treated guinea pigs. This finding was shown microscopically to be due to congestion and hyperplasia of reticulum cells in the red pulp. The liver of one of these guinea pigs showed mild centrolobular vacuole formations which were fat negative; it was assumed that these vacuoles had contained glycogen.

The average of terminal hemograms of the streptovaricin group, which is presented in table 1, showed slight but uniform decreases in the hematocrit and hemoglobin values and the total leukocyte count. In addition, moderate aniso-

TABLE 1

THE EFFECT OF STREPTOVARICIN ON GUINEA PIG HEART BLOOD HEMOGRAMS

	Hematocrit	Hemoglobin	Leukocytes	Neutrophils		Lymphocytes		Monocytes	Eosinophils	Basophils
				Segmental	Nonsegmental	Large	Small			
	per cent	*gm. per 100 ml.*	*10^3/cu. mm.*	*per cent*		*per cent*		*per cent*	*per cent*	*per cent*
Streptovaricin (125 mg. per guinea pig)										
Range	41.0–42.6	11.5–12.2	7.2–15.0	6–10	—	1–5	81–86	2–5	0–5	0–1
Average (4 animals)	41.9	11.8	11.3	9	—	3	83	4	2	<1
The erythrocytes of 2 of 4 guinea pigs showed moderate anisocytosis and polychromasia. Stippled erythrocytes were present in 3 of these 4 guinea pigs.										
Control (0 mg. per guinea pig)										
Range	44.5–47.9	13.8–14.2	13.7–22.8	6–16	—	2–11	68–86	2–3	0–5	0–1
Average (4 animals)	46.2	14.0	18.2	13	—	5	76	3	3	<1

The differential counts were based on 200 cells. The hemograms are based on the 28-day toxicity results.

cytosis, polychromasia, and stippling of erythrocytes of some of these guinea pigs were observed.

Studies with guinea pigs infected with M. tuberculosis: The infection was established as described above, and treatment was initiated at 21 days post infection and was continued through the eighty-third day. During this period two guinea pigs died at 55 and 80 days post infection on the 6.25 mg. level of streptovaricin. Typical tuberculous lesions were observed in both guinea pigs on gross and microscopic examination.

At the eighty-fourth day the surviving animals were sacrificed, and the gross tuberculous involvement was determined. The following observations were made.

Site of inoculation: Guinea pigs of the groups treated with 100 and 50 mg. of streptovaricin were free of cutaneous ulcerations. In 2 guinea pigs, each of which was given 5 mg. of isoniazid and 15 mg. of streptomycin sulfate, recently formed cutaneous scar tissue was present; whereas the other 2 guinea pigs in each of these two groups showed well-healed or inapparent inoculation sites similar to those of the guinea pigs that received 100 and 50 mg. of streptovaricin. Recent scar tissue was observed in all 4 animals given 25 mg. of streptovaricin. Deep irregular ulcers which varied in size up to approximately 2 cm. were present consistently in guinea pigs on the lower two dosage levels (12.5 and 6.25 mg.) of streptovaricin and the two control groups. The site of inoculation was not examined microscopically.

Axillary and superficial inguinal lymph nodes: Using the number of caseated nodes and the amount of caseation as criteria, a moderate degree of involvement was recognized in animals given the upper three dosage levels (100, 50 and 25 mg.) of streptovaricin as well as the isoniazid and streptomycin groups. In these five groups of guinea pigs, no caseation was observed in one to 3 of the animals in each group; the higher figure was recorded for the animals that received 100 mg. of streptovaricin. In guinea pigs on the lower two dosage levels (12.5 and 6.25 mg.) of streptovaricin and in the control groups the gross involvement was regarded as marked.

Lung, spleen, liver: In general, the distribution of gross tuberculous lesions of these organs was restricted to the lower two dosage levels (12.5 and 6.25 mg.) of streptovaricin and to the control groups. In the lungs and liver, typical coalescing tuberculous nodules were observed, whereas grossly the splenic involvement appeared more diffuse and tubercles were less readily discernible.

In addition to the gross observations described above, detailed histologic examination of the tissue obtained at necropsy were made, and it is evident from these data (figure 1), as well as from the gross observations, that streptovaricin is effective in the tuberculous guinea pig as well as in the tuberculous mouse (2). For comparison, the results obtained with streptomycin and isoniazid are indicated in the same figure.

Histopathologic Findings

Axillary and superficial inguinal lymph nodes: Caseated nodules were present in one or more of the lymph nodes, which drain the subcutaneous site of inoculation, of one or more guinea pigs of all treated and control groups. Acid-fast bacilli were consistently observed in areas of caseation necrosis in these lymph nodes and other organs, but they seldom were seen in nodules of epithelioid cells. In those lymph nodes in which the caseated area showed well-defined fibroblastic encapsulation and in which some calcification usually was present, the

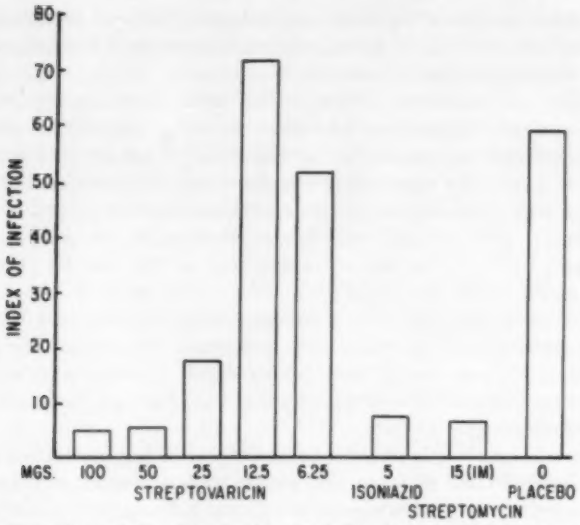

FIG. 1. A comparison of the average index of infection in tuberculous guinea pigs treated with streptovaricin, isoniazid, or streptomycin. Average index of infection in guinea pigs was based on histopathologic characteristics. The index for each animal was determined as suggested by Karlson and Feldman (4) by assignment of value of 10 to 35 for organs with actively progressing tuberculous lesions, depending on the extent of involvement. Values of 1 to 3 were given for healed lesions with fibrosis of calcification and no necrosis.

lesion was regarded as probably nonprogressive. Based upon this interpretation, only in guinea pigs in the group which received 50 mg. of streptovaricin were no progressive tuberculous lesions found. In the group which received 100 mg. of streptovaricin and in the streptomycin group, the nodes of single animals of each group were considered to be probably progressive. In the other streptovaricin groups (which received *25, 12.5, and 6.25 mg.*) and the isoniazid and placebo control groups, progressive tuberculous lesions were present in 3 of 4 of the guinea pigs.

Lungs: The cellular inflammatory response in the lungs was characterized rather uniformly in all groups by focal lymphoid hyperplasia, infiltration of alveolar walls by lymphocytes and mononuclear phagocytes (including epithelioid cells), the formation of epithelioid nodules and, to a lesser degree, by the presence of multinucleated giant cells of the Langhans' type. The septal infiltration of inflammatory cells resulted in an atelectatic and emphysematous pattern which was most often seen in the apical lobes. Caseation and calcification were uncommon findings.

Although the inflammatory pattern was similar in all groups, the tuberculous involvements in the lungs of guinea pigs which received 100 and 50 mg. of streptovaricin and the isoniazid and streptomycin groups were regarded as nonprogressive. The lungs of one or more of the guinea pigs of the remaining groups

of streptovaricin and of the placebo control group showed progressive lesions. These evaluations were based principally on the degree of involvement and the amount of functional lung tissue.

Spleen: The only consistent finding in this organ in guinea pigs of all groups was the conspicuous hyperplasia of reticulum cells, especially in the red pulp. With the exception of the guinea pigs on the 100 and 50 mg. levels of streptovaricin, epithelioid nodules were observed in some animals of the remaining groups. In the isoniazid and streptomycin groups, epithelioid nodules were seen in one and 2 guinea pigs of these groups, respectively; whereas, on the lower dosage levels (25, 12.5 and 6.25 mg.) of streptovaricin and in the placebo control group, epithelioid nodules were present in the majority of the animals. Caseated nodules were found in about one half of the guinea pigs on the 25, 12.5, and 6.25 mg. levels of streptovaricin. Calcific granules were present in one, 3, and one guinea pig, respectively, of those on the 25 and 12.5 mg. levels of streptovaricin and in the isoniazid group. No caseation or calcification was observed in the spleens of the placebo control group.

The splenic lesions of guinea pigs on the 100 and 50 mg. levels of streptovaricin and in the isoniazid and streptomycin groups were evaluated as nonprogressive, whereas one half of the guinea pigs on the 25 and 6.25 mg. levels of streptovaricin and in the placebo control group had progressive tuberculous lesions. On the 12.5 mg. level of streptovaricin, all 4 guinea pigs had progressive lesions.

Liver: Epithelioid nodules were present in 3 of 4 of the guinea pigs on the 25, 12.5, and 6.25 mg. levels of streptovaricin and in the placebo control group.

TABLE 2

A Comparison of the Average Severity of Tuberculous Lesions in Guinea Pigs Treated with Streptovaricin, Isoniazid, or Streptomycin

Treatment	Spleen (Maximum: 35)	Lungs (Maximum: 30)	Liver (Maximum: 25)	Site of Inoculation and Contiguous Lymph Nodes (Maximum: 10)	Average Index of Infection (Maximum: 100)
Streptovaricin (mg. per day)					
100.0	0.75	3.00	0.0	1.25	5.00
50.0	0.25	1.75	0.5	2.75	5.25
25.0	5.75	6.5	1.75	3.75	17.75
12.5	25.0	15.0	21.25	10.0	71.25
6.25	18.33	12.5	13.25	7.25	51.33
Isoniazid (mg. per day)					
5.0	0.25	2.75	0.25	4.0	7.25
Streptomycin (mg. per day)					
15.0	1.5	0.75	0.75	3.5	6.5
Placebo control	11.5	15.25	12.5	10.0	59.25

Evaluation was made with histopathologic key table of Karlson and Feldman (4) on 4 guinea pigs per group.

This lesion was seen in only one guinea pig, each, of the isoniazid and the streptomycin groups, and none were found in animals on the 100 and 50 mg. levels of streptovaricin. Calcified nodules were observed in one guinea pig on the 25 mg. level of streptovaricin and in the guinea pig of the streptomycin group mentioned above. Of the aforementioned liver lesions, only those in the 4 guinea pigs on the 12.5 mg., 2 on the 6.25 mg. level of streptovaricin, and 2 of the placebo control group animals were regarded as progressive.

A numerical value has been assigned to each of these tissues detailed above and these data have been summarized in table 2.

Discussion

These data made it apparent that streptovaricin was efficaceous in the control of experimental tuberculosis in guinea pigs. In the 50 mg. group of guinea pigs, no progressive tuberculous lesions were observed in the lymph nodes which drained the site of inoculation or the lungs, spleen, and liver. In one guinea pig of the 100 mg. group, tuberculous lesions regarded as progressive were present in some of the lymph nodes which drained the site of infection. In other tissues of this group of guinea pigs, slightly fewer lesions than those in the 50 mg. group were observed. It is significant that the site of inoculation was free of cutaneous ulcerations in all guinea pigs of both groups. The indices of infection of the 100 and 50 mg. groups, as shown in table 2, were 5.0 and 5.25, respectively, and compare very favorably with the values of 6.5 and 7.25 obtained in the animals treated with streptomycin and isoniazid.

The 25 mg. dose of streptovaricin was regarded as subeffective inasmuch as progressive lesions were found at the site of inoculation, lungs, and spleen. However, the average index of infection (17.75) was considerably lower than that in the placebo control (59.25), indicating some effect on the infection. The possibility that longer treatment with the lower dose of streptovaricin might have resulted in a better therapeutic effect was not determined.

There are inherent difficulties in evaluation of the data from an experimental infection of this type in guinea pigs. The use of a subcutaneous route of infection does not result in a uniform degree of pathologic involvement. In the placebo control group in which the average index of infection was regarded as somewhat low (59.25), one animal showed fewer lesions than were observed in the other animals of that group. It seems likely that the index of infection of this control group would have been higher if there had been more animals in the group; this seems justified on the basis of gross observations of all 8 of the control animals.

It is of interest that streptovaricin is about 100 times less active than isoniazid in the tuberculous mouse (2), when compared on a weight basis, whereas, in the tuberculous guinea pig that difference is reduced to a factor of 10. The possibility that this represents a difference in the way the two drugs are metabolized or absorbed must be considered, although it is more likely that differences in the mode of action of the drugs accounted for the increased effectiveness of streptovaricin when administered over long periods of time.

Summary

Streptovaricin, a new antimicrobial, has been shown to be therapeutically effective in guinea pigs infected with *Mycobacterium tuberculosis* var. *hominis* (H37Rv).

The antituberculous activity of this antimicrobial was demonstrated with oral doses of 50 and 100 mg. daily by the relative infrequency of progressive lesions in the lymph nodes, lungs, livers, and spleens of infected animals.

In a sixty-two-day study, using the experimental system described above, a 50 mg. dose of streptovaricin had a therapeutic effect comparable with that of 5 mg. of isoniazid or 15 mg. of streptomycin.

Sumario

Estudios con la Estreptovaricina en el Cobayo Tuberculoso

La estreptovaricina, nuevo antimicrobiano, se ha mostrado eficaz terapéuticamente en los cobayos infectados con el *Mycobacterium tuberculosis* var. *hominis* (H37Rv).

La actividad antituberculosa de este antimicrobiano quedó demostrada con dosis orales de 50 y 100 mg. diarios por la relativa infrecuencia de lesiones evolutivas en los ganglios linfáticos, pulmones, hígados y bazos de los animales infectados.

En un estudio que duró sesenta y dos días, usando el sistema experimental descrito más arriba, la estreptovaricina a una dosis de 50 mg. ejerció un efecto comparable al de 5 mg. de isoniacida o de 15 mg. de estreptomicina.

Resume

Etude de la Streptovaricine chez le cobaye tuberculeux

La streptovaricine, un nouvel antimicrobien, a démontré son efficacité thérapeutique chez des cobayes inoculés avec le *Mycobacterium tuberculosis* var. *hominis* (H37Rv).

L'activité tuberculostatique de cet antimicrobien a été démontrée avec des doses orales de 50 et 100 mg. par jour par la rareté relative de lésions évolutives dans les ganglions lymphatiques, les poumons, le foie et les reins des animaux tuberculisés.

Au cours d'une étude de soixante deux jours, dans les conditions expérimentales décrites, la streptovaricine à la dose de 50 mg. par jour produisait un effet thérapeutique comparable à celui de 5 mg. d'isoniazide ou de 15 mg. de streptomycine.

Acknowledgment

The authors wish to acknowledge the advice of Dr. H. R. Reames and the technical assistance of H. Kurtz, J. Kay, D. Johnson, and M. Prestrud.

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POLYSACCHARIDE SKIN TEST ANTIGENS DERIVED FROM HISTOPLASMA CAPSULATUM AND BLASTOMYCES DERMATITIDIS[1, 2, 3]

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(Received for publication October 28, 1957)

INTRODUCTION

The skin test is a widely employed method for the demonstration of past or present infection with respiratory or systemic mycotic invaders. The skin test reagent, histoplasmin, was defined by Zarafonetis and Lindberg (1) as the antigenic substance or substances derived from the mycelial growth of *Histoplasma capsulatum* in broth. The term histoplasmin is currently used to indicate the filtrate derived from the prolonged growth of the mycelial phase of *H. capsulatum* in a liquid medium (2). Unfortunately, it is not always clear from reports of investigators employing histoplasmin skin tests whether the product and the given dilutions are comparable to any other histoplasmin preparation. The National Institutes of Health have partially resolved this problem by preparing standard histoplasmin for comparative studies and by publishing regulations that must be satisfied before a histoplasmin product may be marketed (3). However, similar standardization of the analogous reagents blastomycin and coccidioidin has not been achieved.

The working hypothesis was proposed that, if a known weight of a mycotic skin test antigen could be employed diagnostically, this substance would have the same advantages that PPD has in tuberculosis. The present investigation was conducted with the practical goal of isolating skin test reactive polysaccharide substances from *H. capsulatum* and *Blastomyces dermatitidis*.

EXPERIMENTAL PROCEDURE

Two strains each of *B. dermatitidis* and *H. capsulatum* were used as the organisms for extraction of the polysaccharide substances.

The yeast phase of each of these fungi was grown in 6-liter amounts of tryptose phosphate (Difco) broth enriched with cystine (0.05 per cent), maltose (1.0 per cent), and yeast extract (0.4 per cent).

The flasks were agitated on a slowly moving shaker platform during seven days of incubation at 37° C. The formalin (0.5 per cent) killed yeast-phase cells were separated from the broth by Seitz filtration, and the cells and broth were treated separately for the extraction of the polysaccharides. The method of Heidelberger and co-workers (4) for the isolation of

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[2] This investigation was supported, in part, by research grant E-922 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service; and, in part, by a research grant from the Office of Naval Research.

[3] This paper is taken, in part, from thesis material submitted by R. A. Knight in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School, University of Utah.

pneumococcal specific substances was employed for the extraction of the polysaccharides, except for slight modifications made necessary by local circumstances. In brief, the process consists of the following steps:

1. Precipitation of the polysaccharides from broth or aqueous cell extract by 10 volumes of cold 95 per cent ethanol.
2. Solution of the precipitate in acetate buffered saline, pH 6.8, and removal of protein with chloroform (5).
3. Removal of inorganic ions and other small molecules by dialysis against distilled water.
4. Final precipitation of the polysaccharides with 2 volumes of cold ethanol.
5. Solution of the precipitate in a minimal amount of distilled water and evaporation to dryness by desiccation.

Results

In different experiments, yields of 1.0 to 3.0 gm. of polysaccharides have been obtained from the broth filtrates, while amounts of less than 1.0 gm. have been extracted from the yeast-phase cells of each of three strains of *H. capsulatum* and *B. dermatitidis*. These polysaccharides are gray to light tan in color and are readily soluble in water and saline. The dry powders do not change in appearance when stored in the refrigerator, and the aqueous solutions showed no change in skin test activity after storage at 5° C. for three months.

Data concerning the chemical nature of polysaccharide substances obtained from one strain of each species are presented in table 1. Variations of significance were not noted in analyses of the polysaccharides of the other strains of organisms. The analyses for reducing sugars (6) were performed on polysaccharides hydrolyzed with 6N hydrochloric acid for six hours. This period was chosen on the basis of studies by Miya (7) concerning the structure of a bacterial polysaccharide. The method of Dische and Shettles (8) for pentose and hexose was employed to determine the carbohydrate content after three minutes' and/or ten minutes' incubation with sulfuric acid and the subsequent addition of cysteine for the development of characteristic color. Presumably, the hexose content would approach the reducing sugar content of the polysaccharides if the reaction

TABLE 1

Some Chemical Tests on H. Capsulatum and B. Dermatitidis Polysaccharides

Test	*H. capsulatum* Composition		*B. dermatitidis* Composition	
	Qualitative	Per Cent	Qualitative	Per Cent
Iodine	Negative		Negative	
Molisch	Positive		Positive	
Biuret	Negative		Negative	
Nitrogen		4.00		4.9
Reducing substances* (6 hour hydrolysis)		90.00		75.0
Phosphorus		1.07		0.8
Hexose		58.80		50.0
Pentose	Negative		Negative	

* Includes hexose.

was measurable after long periods of heating in the sulfuric acid. The spectrophotometric sugar color reactions of Dische and Shettles (8), paper chromatography, and micro-Kjeldahl nitrogen determinations indicate that the polysaccharides consisted of hexose and a small amount of nitrogen.

The skin test reactivity of these substances was determined in guinea pigs infected with either *B. dermatitidis* or *H. capsulatum* yeast-phase organisms. One month after the injection of the organisms, the flanks of the animals were clipped and marked off in squares for the injection of known amounts of *H. capsulatum* and *B. dermatitidis* polysaccharides.

The skin test activity of both *H. capsulatum* and *B. dermatitidis* polysaccharides, when injected into the skin of albino guinea pigs infected with *H. capsulatum*, is set forth in table 2. Among 11 animals tested in two experiments, all reacted to 10 γ of polysaccharide prepared from *H. capsulatum* strains G17(10) and G17(M). None of these guinea pigs reacted when injected with saline or 100 γ of *B. dermatitidis* polysaccharides from strains 380 and 410. Standard histoplasmin (National Institutes of Health Lot No. 2) yielded positive reactions of the same magnitude as 10 γ amounts of *H. capsulatum* (broth) polysaccharides. Commercial blastomycin, employed in the designated test strength, showed two positive and four doubtful cross-reactions.

In table 3 are summarized data that were obtained when *B. dermatitidis* poly-

TABLE 2

SKIN TEST REACTIONS OF GUINEA PIGS INFECTED WITH H. CAPSULATUM (G17M)

Albino Guinea Pigs	Polysaccharide (Broth) *H. capsulatum*				Histoplasmin (National Institutes of Health, No. 2)	Blastomycin (Commercial)	Polysaccharide (Broth) *B. dermatitidis*				Saline
	G17(10)		G17M				380		410		
	50 γ*	10 γ	50 γ	10 γ	1:100	1:100	100 γ	10 γ	100 γ	10 γ	
1	+†	+	+	+	+	±	—	—	—	—	—
2	+	+	+	+	+	±	—	—	—	—	—
3	++	+	++	+	+	—	—	—	—	—	—
4	++	+	+	+	+	—	—	—	—	—	—
5	+	+	+	+	+	—	—	—	—	—	—
6	+	+	+	+	+	—	—	—	—	—	—
7	0‡	+	0	+	+	±	—	—	—	—	—
8	0	+	0	+	+	—	—	—	—	—	—
9	0	+	0	+	+	+	—	—	—	—	—
10	0	+	0	+	++	+	—	—	—	—	—
11	0	+	0	+	+	±	—	—	—	—	—
12-20 (controls, noninfected)	—	—	—	—	—	—	—	—	—	—	—

* The amounts indicated, in micrograms of polysaccharide, were intradermally injected in a volume of 0.05 ml. of saline.

† Degree of skin reaction coded as follows:

± = induration from 1 to 4 mm.

\+ = induration from 5 to 10 mm.

++ = induration from 10 mm.

‡ Indicates no test with 50 γ.

TABLE 3

SKIN TEST REACTIONS OF GUINEA PIGS INFECTED WITH B. DERMATITIDIS (380)

Albino Guinea Pigs	Polysaccharide (Broth) *H. capsulatum*				Histoplasmin (National Institutes of Health, No. 2)	Blastomycin (Commercial)	Polysaccharide (Broth) *B. dermatitidis*		Saline
	G17(10)		G17M				380	410	
	50 γ	10 γ	50 γ	10 γ	1:100	1:100	10 γ	10 γ	
1	++*	±	++	±	—	—	+	+	—
2	++	±	++	—	—	—	+	±	—
3	+	±	+	±	±	±	++	++	—
4	++	—	++	—	—	±	+	+	—
5	±	±	—	—	—	±	±	±	—
6	+	±	+	—	—	±	+	+	—
Control (non-infected)									
7	—	—	±	—	—	—	—	—	—
8	—	±	—	—	—	—	—	—	—
9–12	—	—	—	—	—	—	—	—	—

* Degree of skin reaction coded as in table 2.

TABLE 4

HOMOLOGOUS AND HETEROLOGOUS SKIN TEST REACTIONS OBTAINED WITH H. CAPSULATUM POLYSACCHARIDES

Antigen	Amount Injected	Skin Reaction
Guinea Pigs Infected with *H. capsulatum* G17M		
H. capsulatum polysaccharides	50 γ	13*
H. capsulatum polysaccharides	10 γ	10
National Institutes of Health histoplasmin, 1:100 dilution	0.1 ml.	10
Saline	0.05 ml.	0
Guinea Pigs Infected with *B. dermatitidis* 380		
H. capsulatum polysaccharides	50 γ	9.0
H. capsulatum polysaccharides	10 γ	1.6
National Institutes of Health histoplasmin 1:100 dilution	0.1 ml.	0
Saline	0.05 ml.	0

* Mm. (mean) of induration measured 24 hours after injection. All noninfected guinea pigs tested have shown negative reactions to the antigens.

saccharides were injected into guinea pigs infected with *B. dermatitidis*, strain 380, as well as the heterologous skin test reactions resulting from the injection of *H. capsulatum* polysaccharides. It will be noted that cross-reactions obtained with 50 γ of *H. capsulatum* polysaccharide were avoided when 10 γ were employed as the test dose.

The results of the guinea pig skin tests reported in tables 1 and 2 are sum-

TABLE 5

HOMOLOGOUS AND HETEROLOGOUS SKIN TEST REACTIONS OBTAINED WITH B. DERMATITIDIS POLYSACCHARIDES

Antigen	Amount Injected	Skin Reaction
Guinea Pigs Infected with *B. dermatitidis* 380		
B. dermatitidis polysaccharides	100 γ	9.0
B. dermatitidis polysaccharides	10 γ	6.0
Blastomycin, 1:100 dilution	0.1 ml.	2.0
Saline	0.05 ml.	0
Guinea Pigs Infected with *H. capsulatum* G17M		
B. dermatitidis polysaccharides	100 γ	2.0
B. dermatitidis polysaccharides	10 γ	0
Blastomycin 1:100 dilution	0.1 ml.	2.0
Saline	0.05 ml.	0

marized in tables 4 and 5. The average diameters, in millimeters of induration, are given.

DISCUSSION

Skin-reactive substances, isolated by chemical extraction from cultures of *H. capsulatum*, *B. dermatitidis*, and *Coccidioides immitis*, have been reported by other investigators (9–17). It is generally agreed by those who have considered this problem that an ideal skin test antigen at a critical dilution should give a maximal number of homologous responses and a minimal number of heterologous responses; that is, the material should be both sensitive and specific. The question of the specificity of fungal skin test antigens is of prime importance because it has been well established that skin test cross-reactions occur with histoplasmin, blastomycin and, to a lesser degree, with coccidioidin and haplosporangin. This lack of specificity is the rationale for testing humans suspected of having pulmonary mycoses with the three major systemic fungal skin test antigens (histoplasmin, blastomycin, and coccidioidin).

In the present investigation the soluble specific substances derived from the broth rather than from the yeast-phase cells have been employed as skin test antigens because greater yields (up to 3 gm.) have been obtained. Similar skin test reactions were observed with soluble specific substances derived from the cells and broth. These similar skin test reactions obtained with equal amounts of the cell and broth soluble specific substances suggest that the greater quantity of polysaccharides obtained from broths was not due to contaminating artifacts, but may have been due to the release of polysaccharide substances into the medium during growth.

Fifty micrograms of *H. capsulatum* soluble specific substances, contained in 0.05 ml. injected intradermally into guinea pigs infected with *H. capsulatum* produced an average diameter of induration measuring 13 mm. Ten micrograms of this same polysaccharide produce a skin reaction equal to the standard Na-

tional Institutes of Health Lot No. 2 histoplasmin (10 mm.). In the guinea pigs infected with *B. dermatitidis*, the 50 γ of *H. capsulatum* polysaccharide produced an heterologous reaction of 1.6 mm., which is significantly less than a minimum of 5 mm. of edema arbitrarily considered to be a positive reaction.

It is indicated in table 5 that both the 100 γ and 10 γ amounts of *B. dermatitidis* soluble specific substances, each contained in 0.05 ml., produced positive skin reactions, while the commercially available blastomycin did not produce skin reactions of 5 mm. in diameter.

These data suggest that *H. capsulatum* and *B. dermatitidis* infections in guinea pigs may be detected by the use of microgram amounts of polysaccharide antigens.

Summary

Specific soluble polysaccharides have been extracted from yeast-phase broth cultures of *H. capsulatum* and *B. dermatitidis*. Ten micrograms of these skin test polysaccharides have been sufficient to produce skin test reactions in guinea pigs infected with either *H. capsulatum* or *B. dermatitidis*.

Sumario

Antígenos para Cutirreacción de Polisacáridos Derivados del Histoplasma Capsulatum y del Blastomyces Dermatitidis

De los cultivos en caldo del *H. capsulatum* y del *B. dermatitidis* en la fase de levadura se han extraído polisacáridos solubles específicos. Diez microgramos de estos polisacáridos han bastado para producir cutirreacciones en los cobayos infectados ya con el *H. capsulatum* o el *B. dermatitidis*.

Resume

Test cutané avec des antigènes polysaccharides dérivés de Histoplasma capsulatum et de Blastomyces dermatitidis

Des polysaccharides solubles spécifiques ont été extraits de cultures sur bouillon de *H. capsulatum* et de *H. dermatitidis* à la phase levure. Dix microgrammes de ces polysaccharides destinés au test cutané ont été suffisants pour produire des réactions cutanées quand ce test a été pratiqué chez les cobayes inoculés avec *H. capsulatum* ou *H. dermatitidis*.

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TUBERCULOSIS AND DIABETES[1,2]

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(Received for publication September 18, 1957)

Introduction

The purpose of the present paper is to compare the results of the treatment of diabetic tuberculous patients in the years prior to and after the advent of chemotherapy.

In the Philadelphia Survey (1) it was found that 5.3 per cent of active tuberculosis occurred in diabetics less than forty years old, whereas only 1.7 per cent occurred in diabetics more than forty. Wiener and Kavee (2) reported, in their study, that 18.8 per cent of the diabetics with tuberculosis were less than forty years of age. Reaud (3), in his series of 38 diabetic tuberculous patients, found that 23 (60.6 per cent) developed tuberculosis before the age of forty.

The ages of the patients in the present series of cases are shown in table 1. Approximately 48 per cent of the pre-chemotherapy group and 43 per cent of the chemotherapy group developed diabetes before the age of forty years. Approximately 45 per cent of pre-chemotherapy and 39 per cent of chemotherapy groups were less than forty when the diagnosis of active pulmonary tuberculosis was made. Thus, in the present series of tuberculous diabetics, approximately 40 to 45 per cent developed active tuberculosis before the age of forty years.

Material and Methods

The present series was comprised of 57 tuberculous diabetic patients. Of the 57 patients, 29 were treated prior to the advent of chemotherapy, whereas 28 patients received antituberculous drugs for various periods of time. In addition, 13 of the 28 patients who received chemotherapy had adjunctive major thoracic surgery performed.

In the pre-chemotherapy group there were 15 males and 14 females; 27 of the patients were white, one was a Negro, and one was an American Indian. In the chemotherapy group there were 21 males and 7 females. Twenty-four of the patients were white, 3 were Negro, and one was an American Indian.

The ages of the patients on admission to the hospital are shown in table 2. It may be noted that the age distribution of tuberculous diabetics does not correspond with the age at which diabetes prevails when tuberculosis is not a factor. Joslin and associates (4) estimate that approximately 25 per cent of diabetes in the nontuberculous population occurs in patients less than fifty years of age, 25 per cent in patients between the ages of fifty and sixty years, and 50 per cent in the group more than sixty years of age.

The stage of tuberculosis was classified according to National Tuberculosis Association standards as minimal, moderately advanced, and far advanced. Four (13.8 per cent) of the 29 patients in the pre-chemotherapy group were classified as having minimal disease on admission. On the basis of sputum studies, the disease of one of these patients was considered active; 2 patients were considered to have disease of undetermined activity; and the disease of one was considered inactive. Twelve (41.4 per cent) were considered to have moderately

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[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

[2] Presented at the Saranac Lake Medical Society, Saranac Lake, New York, February 5, 1957.

TABLE 1

AGE AT ONSET OF DIABETES AND TUBERCULOSIS

	Onset of Diabetes		Onset of Tuberculosis	
Age	Pre-Chemotherapy	Chemotherapy	Pre-Chemotherapy	Chemotherapy
Less than 40	14 (48.3%)	12 (42.8%)	13 (44.9%)	11 (39.3%)
More than 40	15 (51.7%)	16 (57.2%)	16 (55.1%)	17 (60.7%)

TABLE 2

AGE OF TUBERCULOUS DIABETICS ON ADMISSION

	Pre-Chemotherapy 29 Patients	Chemotherapy 28 Patients
Less than 50 years	17 (58.6%)	21 (75%)
50 to 60 years	5 (17.3%)	4 (14.3%)
More than 60 years	7 (24.1%)	3 (10.7%)

advanced disease; 10 of these had active disease on admission, one had disease of undetermined activity, and the disease of one was considered inactive. Thirteen (44.8 per cent) were considered to have far advanced active disease at the time of admission. There were no minimal cases among the 28 patients in the chemotherapy group on admission. Eighteen (64.3 per cent) of this group had moderately advanced disease which was active in 17 patients and inactive in one, while 10 (35.7 per cent) had far advanced active pulmonary tuberculosis on admission.

OBSERVATIONS

Duration of diabetes: Root (5) has shown that the longer the duration of diabetes, the higher the percentage of patients who will have evidence of tuberculosis. Boucot and associates (1), in the Philadelphia Survey, found a much higher incidence of active tuberculosis when diabetes had existed for more than ten years. This finding was not apparent in the present pre-chemotherapy group. Eighteen (62.1 per cent) of the 29 patients in this group had diabetes diagnosed first, while 7 (24.1 per cent) had the two diseases diagnosed simultaneously, and 4 (13.8 per cent) had tuberculosis diagnosed first. Eleven (61.6 per cent) of the 18 patients in whom diabetes was diagnosed first had diabetes for less than five years prior to the diagnosis of tuberculosis, whereas 7 (38.9 per cent) had diabetes for more than five years.

In the chemotherapy group, 20 (71.4 per cent) had the diagnosis of diabetes made first, 6 (21.4 per cent) had the two diseases diagnosed simultaneously, and 2 (7.2 per cent) had tuberculosis diagnosed first. Of the 20 who had diabetes diagnosed first, 7 (35 per cent) had diabetes less than five years prior to the diagnosis of tuberculosis, whereas 13 (65 per cent) had diabetes for more than five years prior to the onset of tuberculosis. This is more in agreement with the findings of Root (5) and Boucot and associates (1).

Severity of diabetes: Arbitrarily, the severity of diabetes was classified according to the total daily dose of insulin required to control the diabetes. The types

of insulin used were regular insulin, protamine zinc insulin, globin insulin, and the neutral Protamine Hagedorn type. The severity of diabetes was classified as mild in those patients requiring no insulin, moderately severe in those taking 10 to 40 units of insulin per day, and severe in those taking more than 40 units of insulin daily. In the Philadelphia Survey the highest incidence of active tuberculosis was found in the group of patients less than forty years of age and taking more than 40 units of insulin. In this group, 8.8 per cent had active tuberculosis as compared with 2.6 per cent of active tuberculosis found in the whole group of 3,106 diabetics (1). On the other hand, Reaud (3) found that there was no relationship between the severity of diabetes and the clinical evolution of tuberculosis in his group of 38 patients. Wiener and Kavee (2) also found no obvious relationship existing between the severity of diabetes and the stage of tuberculosis.

In both the pre-chemotherapy and chemotherapy groups in the present study, there was no definite relationship between the severity of the diabetes and the stage of tuberculosis; e.g., there were mild diabetics with advanced tuberculosis and patients with minimal tuberculosis who had moderately severe or severe diabetes. It did appear, however, that, if the diabetes was moderately severe or severe, the tuberculosis was more apt to be in an advanced stage.

Jacobs and associates (6), in their series of 55 tuberculous diabetics, believed that the control and severity of diabetes were separate but related factors. It was their impression that the final outcome of the tuberculosis did not depend as much on the severity of the diabetes as on the effectiveness of its control. Because of insufficient data in the present series, no attempt was made to ascertain whether or not a correlation existed between the control of the diabetes and the evolution of the tuberculous process.

Change in insulin requirements: In the pre-chemotherapy group, there was no significant change in the insulin requirements at discharge as compared with the requirements on admission. In the chemotherapy group this finding was observed in 19 patients, while the insulin requirement was significantly decreased in 8 patients and significantly increased in one patient. Only 2 of the 13 patients who had major thoracic surgery as well as chemotherapy required substantially more insulin in the immediate postoperative period than in the preoperative period; the remaining 11 had approximately the same insulin requirements immediately before and after surgery.

History of coma: Root and Bloor (7) found that 17.8 per cent of those patients admitted in diabetic coma showed evidence of active tuberculosis in five years. Joslin (4) reported that 20 (12.5 per cent) of 159 patients who recovered from diabetic coma eventually died of tuberculosis. No evidence of tuberculosis was found in 66 patients who died in diabetic coma.

In the pre-chemotherapy group there was a history of diabetic coma in 3 patients. Diabetic coma preceded the diagnosis of tuberculosis in 2 patients by intervals of three to six years. In one case tuberculosis was first discovered at the time the patient was in diabetic coma. All 3 patients died of tuberculosis. Three other patients were in acidosis on admission to the hospital and all eventually

TABLE 3

STATUS OF TUBERCULOSIS AT DISCHARGE OR LAST FOLLOW-UP OBSERVATION IN 29 PATIENTS OF PRE-CHEMOTHERAPY GROUP

Minimal, inactive	4
Moderately advanced	11
A. Active	7
Dead—6	
No follow-up—1	
B. Activity undetermined	1
No follow-up—1	
C. Inactive	3
Far advanced, active	14
Dead—14	

died of tuberculosis. In the chemotherapy group one patient gave a history of diabetic coma ten years prior to the diagnosis of tuberculosis. She received what is now considered to be ineffective chemotherapy and eventually died of tuberculosis. Three other patients who were in acidosis on admission also had active tuberculosis at that time. All 3 received adequate chemotherapy; their disease became inactive and has remained in that status.

Comparison of End Results of Treatment in Pre-Chemotherapy and Chemotherapy Eras

The status of the patients in the pre-chemotherapy era at the latest follow-up study is shown in table 3. Four patients were discharged with minimal inactive tuberculosis. One patient was considered to have inactive disease at the time of discharge, and 2 patients showed no signs of activity of their tuberculosis two and three years after discharge. There has been no further follow-up observation in these 3 cases. A fourth patient is considered to have had inactive disease for nineteen years after discharge. Eleven patients were considered to have moderately advanced disease at discharge. Seven were considered to have active disease at the last examination. Six of these are dead; 2 died of active tuberculosis, a third died of a gastrointestinal hemorrhage one year after discharge, and a fourth died of unknown cause ten years after discharge. A fifth patient died of osteomyelitis and the sixth of a myocardial infarction. No further follow-up information is available on the seventh patient. One patient was discharged with moderately advanced tuberculosis of undetermined activity, but there has been no further information on this patient. Three patients were considered to have inactive moderately advanced tuberculosis on the last follow-up study. One patient, who was considered to have had inactive tuberculosis for seven years after discharge, died one month after the last examination, probably of a cardiac condition. Two patients have shown no signs of tuberculous activity for nine and seventeen years after their discharge from the hospital. Fourteen patients were considered to have had far advanced active pulmonary tuberculosis at discharge. All are dead; 13 from tuberculosis and one from carcinoma of the liver.

TABLE 4

STATUS OF TUBERCULOSIS AT DISCHARGE OR LATEST FOLLOW-UP OBSERVATION IN 28 PATIENTS IN CHEMOTHERAPY GROUP

Less than 100 days treatment	7 patients (25%)
6.....dead	
4—far advanced active tuberculosis	
2—other causes	
1.....living and well	
12-18 months' treatment	8 patients (28.6%)
2.....dead—other causes; inactive tuberculosis	
6.....living	
More than 18 months of treatment	13 patients (46.4%)
0.....dead	
13.....inactive	

The status of the chemotherapy group at the last examination is shown in table 4. Seven patients received less than one hundred days of chemotherapy. One is living and well, whereas 6 are dead; 4 died of far advanced active pulmonary tuberculosis and 2 of other causes.

At the time when the 2 patients died of other causes, the tuberculosis was considered active in one and inactive in the other. Eight patients received chemotherapy for twelve to eighteen months. Two who died of other causes were considered to have inactive tuberculosis at the time of death. Six patients are living; 3 of these are well and show no sign of activity of the tuberculous process to date. A fourth patient, who had a single culture positive for tubercle bacilli forty-eight months after discharge, was considered to be well fifty-eight months after discharge. There have been no further follow-up findings in the cases of the fifth and sixth patients who, because of persistent cavitation in one and failure to complete treatment in the other, were considered to have active disease at the the time of discharge, although the sputum examination in both cases was negative for tubercle bacilli on concentrate and culture. Thirteen patients had chemotherapy for more than eighteen months. At the last follow-up observation all were living, and 11 are considered to have definitely inactive disease. One patient left against advice and was admitted shortly thereafter to another hospital where a sputum culture positive for tubercle bacilli was obtained. Six and again eighteen months later he was considered to have arrested disease. The thirteenth patient, who was considered to have inactive disease at discharge, was re-started on drugs at another clinic five months after discharge, and drugs were being continued nineteen months after discharge, although his disease was classified as inactive.

A summary of the above results is recapitulated in table 5. Eight (27.5 per cent) of the 29 patients in the pre-chemotherapy group were living at the time of the latest examination and 21 (72.5 per cent) were dead. Fifteen of these latter died of active pulmonary tuberculosis and 6 of other causes. Five of the 6 patients who died of other causes had active tuberculosis at the time of death. In this group there was a mortality rate of 51.7 per cent due to active pulmonary tuber-

TABLE 5

STATUS OF TUBERCULOSIS AT DISCHARGE OR LAST FOLLOW-UP OBSERVATION

	Pre-Chemotherapy 29 Patients	Chemotherapy 28 Patients
Living	8 (27.5%)	20 (71.4%)
A. Minimal, inactive	4	1
B. Moderately advanced	4	13
Activity undetermined	1*	0
Inactive	1†	11
Active at discharge	2‡	2
C. Far advanced	0	6
Inactive	0	6
Dead	21 (72.5%)	8 (28.6%)
A. Active pulmonary tuberculosis	15 (51.7%)	4 (14.3%)
B. Other causes	6§	4‖

* No further follow-up study.

† For 17 years following discharge.

‡ Disease in one patient inactive 9 years later; no further follow-up study on the second patient.

§ Five had active tuberculosis at the time of death.

‖ Three had inactive tuberculosis and one had active disease at the time of death

culosis. The over-all mortality rate from active pulmonary tuberculosis among nondiabetic tuberculous patients who did not receive chemotherapy during the same interval of time (1936–1949) was 6.7 per cent.

Twenty (71.4 per cent) of the 28 patients who received chemotherapy were living at the time of the latest examination. One was considered to have minimal inactive tuberculosis. Eleven patients were believed to have moderately advanced inactive disease, and 2 were considered to have moderately advanced active disease, one because of persistent cavitation and the other because of failure to finish the prescribed course of chemotherapy. Six were believed to have far advanced, inactive tuberculosis. Eight patients (28.6 per cent) are dead; 4 (14.3 cent) died of far advanced active pulmonary tuberculosis and 4 of other causes. The 4 patients who died of far advanced active pulmonary tuberculosis had received only short-term chemotherapy. Three of the 4 patients who died of other causes were considered to have inactive tuberculosis at the time of death, whereas the disease in the fourth patient was active. Thus, in the chemotherapy group there was a mortality rate of 14.3 per cent due to active pulmonary tuberculosis.

It may be noted that all of the deaths from active pulmonary tuberculosis occurred in the group which received chemotherapy for less than one hundred days. In those patients who received what is now considered to be effective chemotherapy, there were no deaths due to active pulmonary tuberculosis. In the same period of time that was covered by the chemotherapy group (1947–1956), the mortality rate from pulmonary tuberculosis among nondiabetics was 7.4 per cent. This included nondiabetic tuberculous patients who received the benefits of drug treatment as well as some who did not receive chemotherapy.

Surgery

Thirteeen of the 28 diabetic tuberculous patients who received chemotherapy underwent major thoracic surgery. Ten were men; 3 were women. Their ages at the time of the diagnosis of tuberculosis ranged from nineteen to forty-nine years. Nine were considered to have moderately advanced disease and 4 to have far advanced disease. There were no cases of minimal disease. All were considered to have active disease on admission. At that time one was considered to have mild diabetes, 5 moderately severe diabetes, and 7 severe diabetes. In all cases the sputum was positive for tubercle bacilli, and all of the patients had definite evidence of cavitation at some time prior to surgery. One patient had no chemotherapy prior to thoracoplasty. Eight months after surgery he was given a short course of chemotherapy. The other 12 patients received antituberculous chemotherapy for periods ranging from seven to thirty months prior to surgery. A total of twenty surgical procedures were carried out. Twelve pulmonary resections were performed, one patient undergoing bilateral resection. Four thoracoplasties were done which failed to control the disease in 2 patients. Subsequently, pulmonary resections were performed on these 2 patients with good results. Four tailoring thoracoplasties were performed.

At the time of surgery, 7 patients had sputum negative for tubercle bacilli, whereas in 6 the sputum examinations were positive. Five of the 6 who had infectious sputum at the time of surgery had organisms that were resistant to streptomycin, para-aminosalicylic acid (PAS), or isoniazid, either singly or in combination. One of the 5 with resistant organisms had a thoracoplasty performed and 4 had pulmonary resections. One of the 4 who had a pulmonary resection was given pyrazinamide-PAS; PAS, to which the bacilli were susceptible was added to the regimen of a second; a third received pyrazinamide-isoniazid; the fourth received no additional chemotherapy. All patients did well following surgery.

These 13 patients who received both chemotherapy and surgery in order to control their tuberculosis have been followed for periods of time ranging from two to fifty-eight months after discharge. Two patients who died of other causes were considered to have had inactive tuberculosis at the time of death. Eleven of the patients were living and well at the time of the latest follow-up observation. One, who had a culture positive for tubercle bacilli one month after discharge, was considered to have arrested tuberculosis eighteen months later. The second, whose disease was considered to be inactive at discharge and is still considered inactive, was re-started on drugs five months after discharge at another clinic and was still taking drugs nineteen months after discharge. The third had a single culture positive for tubercle bacilli forty-eight months after discharge, but is considered to have inactive disease fifty-eight months after discharge. The remaining 8 patients are considered to have definitely inactive disease.

Comment

The mortality rate from active pulmonary tuberculosis among the diabetic tuberculous group treated at this hospital prior to the advent of chemotherapy

was 51.7 per cent as compared with a mortality rate of 6.2 per cent among the nondiabetic tuberculous patients treated during the same interval of time (1936–1949).

The mortality rate from active pulmonary tuberculosis during the chemotherapy era was 14.3 per cent in the diabetic tuberculous group as compared with 7.4 per cent in the nondiabetic tuberculous group treated during the same period of time. This 7.4 per cent figure is the over-all mortality rate from tuberculosis among nondiabetics, and includes patients who received chemotherapy as well as some who did not. All of the deaths from pulmonary tuberculosis in the diabetic tuberculous group occurred in those who received what is now considered to be ineffective therapy (one hundred days or less).

From this study it appears that the complication of diabetes mellitus in persons afflicted with pulmonary tuberculosis does not appreciably affect the mortality rate or the achievement of an inactive status of their pulmonary disease provided that the individual receives effective chemotherapy as well as appropriate surgical therapy when indicated.

Summary

The mortality rate among diabetic tuberculous patients treated prior to the advent of chemotherapy was eight times that of the nondiabetic group. In the chemotherapy group the mortality rate of diabetic tuberculous patients was approximately twice that of the nondiabetic group.

All of the deaths from pulmonary tuberculosis among the diabetic tuberculous patients treated with chemotherapy occurred in those who received what is now considered to be ineffective therapy. There were no deaths from active pulmonary tuberculosis among those who received effective chemotherapy.

Tuberculous diabetics who received chemotherapy tolerated major thoracic surgery as well as the nondiabetic group.

From this series it appears justifiable to conclude that the mortality rate from pulmonary tuberculosis of diabetic tuberculous patients who receive adequate chemotherapy should be no greater than that of the nondiabetic tuberculous group.

Sumario

Tuberculosis y Diabetes

La mortalidad entre los tuberculosos diabéticos tratados antes del advenimiento de la quimioterapia fué ocho veces mayor que en el grupo sin diabetes. En el grupo quimioterapéutico, la mortalidad de los tuberculosos diabéticos fué aproximadamente el doble que la de los grupos sin diabetes.

Todas las muertes debidas a tuberculosis pulmonar entre los tuberculosos diabéticos que recibieron quimioterapia recayeron en los que recibieron lo que hoy día se considera terapéutica ineficaz. No hubo muertes debidas a tuberculosis pulmonar activa entre los que recibieron quimioterapia eficaz.

Los diabéticos tuberculosos que recibieron quimioterapia toleraron la cirugía torácica mayor tan bien como el grupo sin diabetes.

A juzgar por esta serie, parece justificado deducir que la tasa de mortalidad debida a la tuberculosis pulmonar en los tuberculosos diabéticos que reciben quimioterapia no debería ser mayor que la del grupo tuberculoso sin diabetes.

Resume

Tuberculose et Diabète

Dans cette série de cas, le taux de mortalité parmi les tuberculeux atteints de diabète traités avant l'instauration de la chimiothérapie était huit fois plus grand que celui du groupe non diabétique. Parmi le groupe soumis à la chimiothérapie, le taux de mortalité des tuberculeux diabétiques était approximativement le double de celui des groupes non diabétiques.

Tous les déces par tuberculose pulmonaire chez les tuberculeux diabétiques soumis à la chimiothérapie se sont produits dans les cas ayant reçu ce que l'on considère aujourd'hui une chimiothérapie inefficace. Il n'y eut pas de décès par tuberculose pulmonaire évolutive parmi les malades soumis à une chimiothérapie appropriée.

Les tuberculeux diabétiques recevant la chimiothérapie ont toléré les interventions chirurgicales thoraciques majeures aussi bien que le groupe non diabétique.

Selon cette série, il paraît juste de conclure que le taux de mortalité par tuberculose pulmonaire des tuberculeux diabétiques recevant une chimiothérapie adéquate ne devraît pas être plus élevé que celui du groupe non diabétique.

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Notes

EXPERIMENTS ON THE PROPHYLAXIS OF A MINIMAL TUBERCULOUS INFECTION OF GUINEA PIGS WITH AN INTERMITTENT ISONIAZID REGIMEN[1]

In a letter to the Editor of this journal (1956, *74*, 475), the present writers mentioned experiments showing that a minimal infection in guinea pigs could be prevented by the intermittent administration of isoniazid. A preliminary report of these experiments follows:

The animals were infected subcutaneously in the right thigh with strain Washington I, which is highly pathogenic for guinea pigs and is isoniazid susceptible. Some animals received 10^{-6} mg. (approximately 20 viable units); others were given 10^{-7} mg. (approximately 2 viable units). Five guinea pigs infected with 10^{-7} mg. and 4 guinea pigs infected with 10^{-6} mg. served as controls and were killed after three months. They showed a generalized tuberculosis with extensive involvement of spleen, liver, and lungs. The remaining 40 animals were divided into the following groups:

Group I: 10 animals, infected with 10^{-7} mg. treated with isoniazid, 5 mg. per kg. subcutaneously, twice a week.

Group II: 10 animals, infected with 10^{-6} mg. treated with the same dose as *Group I*.

Group III: 10 animals, infected with 10^{-7} mg. treated with isoniazid, 10 mg. per kg. subcutaneously, twice a week.

Group IV: 10 animals, infected with 10^{-6} mg. treated with the same dose as *Group III*.

The animals were maintained individually in separate cages. Once a week each animal was weighed and the site of infection and the regional lymph nodes were palpated. Therapy was started two hours before infection. The dosage of isoniazid was corrected weekly for weight changes.

The therapy was interrupted after four months in every other animal of each group. This group of guinea pigs was then observed for an additional four months after terminating the therapy, and was then killed if a tuberculous infection of the lymph nodes was not apparent before that time. The regional lymph nodes of the popliteal area and of the groin, one half of the spleen, and a portion of liver and lung from the animals free of macroscopically evident tuberculosis were transplanted subcutaneously in tuberculin-negative guinea pigs. The recipients were killed three months after transplantation and were inspected macroscopically. In some instances remaining portions of organs from the treated animals were ground and cultured on Hohn-egg medium.

The remaining animals were treated for eight months except for 2 guinea pigs which died for other reasons in the eighth month of treatment. The remaining 18 animals were divided in two subgroups. The first group was killed three and one-half months after the end of therapy; the second is still under observation at this time. Half of the spleen of 7 animals of the first subgroup and an equal volume of the lung and of the popliteal, cubital, iliac, portal, and bronchial lymph nodes were transplanted in the neck of recipient animals. Furthermore, 4 cultures on Hohn-egg slants and 2 in Kirchner medium were made of all mentioned tissues.

At certain intervals, the tuberculin reaction was tested intracutaneously in all treated animals with 0.1 ml. of Old Tuberculin (OT) diluted with physiologic saline, 1:5. The reaction was called positive if (*1*) a swelling of at least 10 mm. in diameter was found forty-eight hours after injection; (*2*) the skin of the edematous region elevated with thumb and second finger reached a thickness of at least 4 mm.; and (*3*) an induration the size of a small pea could be palpated after four to six days.

[1] The experiments will be published in greater detail in the *Beiträge zur Klinik der Tuberkulose und spezifischen Tuberkulose-Forschung*, 1957, *116*, 687.

Serial sections were cut of each organ (spleen, liver, lungs, and lymph nodes) of those animals in every treated group which were macroscopically negative for tubercle bacilli at autopsy.

As noted in the former studies,[2a,b] an abscess formed at the site of injection if the infecting dose was 10^{-3} despite treatment with 10 mg. of isoniazid six times a week. In the present experiment, with an infecting dose of 10^{-6} and 10^{-7} mg., no abscess or swelling could be found at the site of inoculation during the period of treatment. In 15 of the 40 treated animals, however, swelling of the lymph nodes in the groin appeared five to ten weeks after infection and vanished two to three weeks later. These palpable nodes were quite firm. No reaction was observed in the nodes of the other groin. This lymphadenopathy is believed to be a component of an abortive primary complex. The tuberculin reactions became positive relatively late and generally were only weak. After three and one-half months, 60 per cent of the animals became reactors; and after seven and one-half months, only one of the 40 treated animals remained a nonreactor. This one was infected with 10^{-6} mg. Despite the very low number of organisms in the inoculated dose of 10^{-7} mg., the tuberculin reaction indicates that an infection was established in all animals.

Details concerning the animals which died or were killed during the experiment are tabulated in table 1. It may be seen that among the animals treated for four months, 10 of 20 (50 per cent) developed tuberculosis. Those guinea pigs which showed tuberculosis were distributed equally among the various groups. A clear-cut relationship between the extent of disease, the infecting dose, and the dose of isoniazid could not be established. The tuberculosis which developed in 4 animals was equal to that in the control (untreated) animals after infection. In 2 other animals the disease also extended from the primary complex, but did not generalize to the same degree as in the controls. In 3 animals at the end of the four-month period after treatment, evidence of disseminated disease was detected only at autopsy. Various lymph nodes and isolated foci were seen, in some of which the tuberculous nature was established by histology and culture only. In one animal a microscopic lesion was found in the lung. The last 10 animals were macroscopically and microscopically free of such foci, and the transplantations of tissues and the cultures were negative. Two of the 20 animals which should have been treated for eight months died seven and one-half months after infection. They were free of tuberculosis, and the transplantations of their tissues remained negative for tubercle bacilli.

The other 18 guinea pigs were divided after the termination of treatment in two groups of 8 and 10 animals. It was intended that the former should be observed for a further three and one-half months; the latter, as long as possible.

One animal in each group showed enlarged tuberculous lymph nodes in the groin shortly after the end of treatment. These 2 animals were then killed. The tuberculosis was restricted to the regional nodes. Therefore, three and one-half months after therapy, 7 remaining animals were autopsied. They all were macroscopically free of tuberculosis, but microscopically, in one animal, a lesion was found in the liver. All transplantations of tissues and all cultures remained negative for *M. tuberculosis*. Two of 8 animals in this group became ill. The remaining 9 guinea pigs in the other groups treated for eight months are still under observation at this time. (It is now eight months since therapy had been stopped.) The animals are well. Although the palpable lymph nodes are unchanged, the tuberculin reaction is now positive. Ordinarily, untreated guinea pigs infected with about

[3] BARTMANN, K., *et al.*: *a*. Beitr. Klin. Tuberk., 1955, *115*, 79; *b*. Beitr Klin. Tuberk., 1956, *115*, 269; *c*. Beitr. Klin. Tuberk., 1957, *117*, 334.

TABLE 1

DATA CONCERNING ANIMALS WHICH DIED OR WERE KILLED DURING EXPERIMENT

(Except Nos. 608 and 613 Which Died Intercurrently for Other Reasons)

Animal	Infecting Dose	Isoniazid Dose	Months of Therapy	Death		Extent of Tuberculosis	Lymph Nodes Palpable After Therapy
				By	Time		
	mg.	mg/kg					
602	10^{-7}	5	4	Sacrifice	4 months after therapy	Portal lymph node only	No
610	10^{-7}	5	4	Sacrifice	4 months after therapy	Portal lymph node and lung	No
621	10^{-7}	10	4	Sacrifice	4 months after therapy	Generalized; less than controls	3¾ months
622	10^{-7}	10	4	Sacrifice	3½ months after therapy	Generalized; as in controls	2½ weeks
623	10^{-7}	10	4	Sacrifice	3½ months after therapy	Generalized; as in controls	7½ weeks
630	10^{-7}	10	4	Sacrifice	3½ months after therapy	Generalized; as in controls	2½ weeks
619	10^{-6}	5	4	Sacrifice	3½ months after therapy	Generalized; less than controls	4 weeks
616	10^{-6}	5	4	Sacrifice	4 months after therapy	Focus in the lung	No
631	10^{-6}	10	4	Natural	2½ months after therapy	Iliacal a. paraaortal lymph nodes only	No
640	10^{-6}	10	4	Natural	2½ months after therapy	Generalized; as in controls	7 weeks
605	10^{-7}	5	8	Sacrifice	3½ months after therapy	Liver only	No
609	10^{-7}	5	8	Sacrifice	7½ weeks after therapy	Regional nodes only	6½ weeks
615	10^{-6}	5	8	Sacrifice	2 months after therapy	Regional nodes only	7½ weeks
608	10^{-7}	5	8	Natural	7½ months after infection	No	No
613	10^{-6}	5	8	Natural	7½ months after infection	No	No

20 viable units of this challenge strain will die in eight months. About 75 per cent of the animals treated for eight months did not develop manifest tuberculosis.

Tubercule bacilli were isolated from several animals with generalized or localized tuberculosis and were tested for isoniazid susceptibility and for pathogenicity for guinea pigs. Both of these properties had remained unchanged.[2a]

* * *

At the outset it should be emphasized that the experiment was performed with a strain of *M. tuberculosis* highly pathogenic for guinea pigs. This strain, although inhibited on egg slants by 0.04 to 0.05 γ of isoniazid per ml., produces manifest tuberculosis in animals after an infection with 10^{-2} mg., even when the animals are treated for three months, six times weekly with 10 mg. of isoniazid per kg. or with isoniazid (10 mg. per kg.) plus streptomycin (20 mg. per kg.) plus PAS (0.2 gm. per kg.) subcutaneously.[3]

In the present experiment which employed a minimal infecting dose, 50 per cent of the guinea pigs were protected against overt disease by an intermittent isoniazid regimen of four months' duration and 75 per cent remained well when treated for eight months. Thus, the protection afforded by isoniazid was not as impressive as in the experiments of Palmer and associates.[4] Real prophylaxis in the sense of preventing the first steps of infection did not result. The animals became tuberculin reactors, and some responded with a temporary enlargement of the regional lymph nodes. It is believed, therefore, that the results indicate a therapeutic arrest of the disease rather than prophylaxis. The term "arrest of the disease" and not "arrest of the infection" is used intentionally. Obviously, the infection in the animals free of tuberculosis did not seem to be a "dormant" one, as defined by McCune, Tompsett, and McDermott,[5] because all of the tissue transplantations and cultures in the present study remained negative for tubercle bacilli, and the technique employed had been highly efficient in previous experiments in this laboratory. Naturally, a "latent" infection cannot be excluded, especially with regard to the tuberculin reaction.

The fact also cannot be excluded that immune reactions are important for the result despite the lack of immunity in the experiments with a minimal infection in mice by Dubos and Schaefer,[6] Robson and Didcock,[7] or Jespersen and Rasmussen.[8] An early tuberculin reaction and an enlargement of the lymph nodes was noted more frequently in animals without manifest disease than in those which became sick. This is demonstrated by the following data:

The tuberculin reaction tested three and one-half months after infection was positive in 17 of the 25 animals with no overt disease, but in only 5 of the 13 ill guinea pigs. An enlargement of lymph nodes could be found in 12 of the 25 animals with no overt disease, but only in 2 of the 13 ill guinea pigs. These differences are statistically important (P between 0.1 and 0.05) with regard to the tuberculin reaction and they are significant ($P = 0.05$) with respect to the enlargement of the glands.

Therefore, in the apparently healthy group two signs frequently correlated with immunity are found more often, namely, allergy and a reaction in regional lymph nodes which seem to be an abortive form of the primary complex. It is hoped to settle this question by experiments with superinfections now under way.

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How should the present results be evaluated, irrespective of which factors are responsible for the successful prevention of the disease? One method for evaluating these results is by comparison with BCG vaccination. This comparison shows that the isoniazid prophylaxis is at least equal to BCG vaccination in guinea pigs. Regarding the degree of protection given by BCG vaccine against a minimal infection, it must be concluded that the development of manifest tuberculosis could be prevented in only a limited number of animals at best.[9-17]

In some of the experiments cited from the literature, the challenge strain was not even highly virulent, and investigations to detect viable challenge bacteria in the protected animals were not carried out in a single case. In the present prophylaxis experiment with isoniazid, it was not possible to detect overt or dormant tuberculosis in 50 per cent (or 75 per cent) of the animals despite challenge with a highly virulent strain and despite a search for germs capable of multiplying. Improvement of the results by a prolongation of the treatment or a more frequent dosage remains to be established.

What effect could be expected by such prophylaxis in man? It is believed that the results might be better than those in guinea pigs. Since isoniazid dosages for an adequate treatment of tuberculosis in man and in guinea pigs are the same, it would be expected that at least an arrest of the disease in man would be obtained. Humans also develop acquired immunity, and man's native resistance is greater than that of guinea pigs. Therefore, the bacteria suppressed in their multiplication by isoniazid would probably be destroyed more quickly in the human than in the guinea pig. And, even if the bacteria are not rapidly destroyed in man, it should be possible by an intermittent isoniazid regimen to protect a human being during the critical phase in which his own resistance is insufficient to withstand the infection. This seems to the present writers to be the minimum that may be expected, and it justifies isoniazid prophylaxis in man. The question of which groups of persons should receive prophylaxis is not germane to the present discussion.

Protection by BCG in animals is relatively short,[13, 18] and its duration in man is uncertain and unpredictable in the individual case. It may be assumed that prophylaxis is effective as long as it is administered, provided the infection is not caused by resistant germs or that the bacteria do not become resistant. Infection with isoniazid-resistant germs is still so rare as to be of no consequence in a consideration of prophylaxis.[19, 20] As far as is known, conversion from drug susceptibility to resistance is independent of the drug treatment employed because chemotherapy seems to select only pre-existing resistant mutants.[21] There is a low probability for a mutation if—as is usual in a natural infection—only a few bacteria are present. Therefore the possibility of developing drug resistance during prophylaxis is negligible, but already established resistance would naturally make it ineffective.

The question arises whether more frequent administration of isoniazid would give

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[18] BLOCH, H., AND SEGAL, W.: Am. Rev. Tuberc., 1955, *71*, 228.
[19] CHAVES, A. D., *et al.*: Am. Rev. Tuberc., 1955, *72*, 143.
[20] MEISSNER, G.: Beitr. Klin. Tuberk., 1956, *13*, 57.
[21] HEILMEYER, L.: Ergebn. ges. Tuberk.—Forsch., 1956, *57*, 107.

better protection. According to the experience of the present writers with adults, some difficulties often arise in persons taking the drug only twice a week when they are not on bed rest. The personnel of this department have taken prophylactic isoniazid voluntarily, 5 mg. per kg. twice a week for the last several months. Some persons could not tolerate this dose despite their good will and cooperation. This fact is in agreement with the experiences of Auersbach and Grunze[23] on isoniazid therapy of outpatients. For more widespread prophylactic use, it would be advisable to develop better tolerated isoniazid derivatives. Be that as it may, the prospect for better protection by daily isoniazid administration does not seem very good, because it has been noted that 10 mg. per kg. of isoniazid twice a week in guinea pigs with generalized tuberculosis gives results only slightly inferior to a schedule of 10 mg. per kg. six times weekly (unpublished results).[23] It remains to be investigated if a combination of isoniazid prophylaxis and BCG vaccination would be synergistic. The experiments published by Mitchison and Selkon[24] have shown that isoniazid treatment was improved by BCG vaccination as determined by enumeration of viable bacteria in the spleen. Furthermore, the experiments of Jespersen and Rasmussen suggest a synergistic effect of isoniazid administration and BCG vaccination.

In summary: Guinea pigs were infected with a few tubercle bacilli of the highly virulent human strain Washington I. Treatment with isoniazid, 5 or 10 mg. per kg. every three or four days, was started simultaneously with infection and was maintained in one group for four months and in another group for eight months. The first group was observed for four months after treatment and then examined at autopsy. In all animals free from tuberculosis observed macroscopically, an attempt was made to find viable bacteria in various organs by culture and animal inoculation. The tissues were also investigated histologically. In one-half of the animals of the first group it was not possible to demonstrate tuberculous changes or culturable tubercle bacilli. One portion of the animals of the second group were killed three and one-half months after treatment and were investigated as were the animals in the first group. The other portion remained alive and have been under observation for eight months and seem to be healthy. In about three-fourths of the second group, tuberculous changes or culturable tubercle bacilli could not be demonstrated.

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[23] Auersbach, K., and Grunze, H.: Deutsche med. Wchnschr., 1953, *78*, 1767.

[23] Since this manuscript went to press, the results of further experiments have become available. The results indicate that a continuous dosage of isoniazid gives substantially better results than a regimen of intermittent dosage.

[24] Mitchison, D. A., and Selkon, J. B.: Am. Rev. Tuberc., 1956, *74* (Supplement, p. 109).

A RAPID MOUSE TEST FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

I. The Enhancement of Experimental Tuberculosis in Mice by Hog Gastric Mucin

The use of hog gastric mucin as an agent to enhance experimental infections in laboratory animals (mice, in particular) has been very extensive with a large variety of microorganisms, including bacteria, fungi, and viruses.[1] Other mucins of diverse origins, such as those derived from the human respiratory tract,[2, 3] human stomach,[2, 4] human intestines,[2] hog intestine,[5] guinea pig intestine,[6] and mouse stomach[7] have also been found to be "virulence enhancing." However, very little work has been published on the enhancement of infection with *Mycobacterium tuberculosis* by hog gastric mucin or any other mucin. Mills and Colwell[8] reported enhanced local infection in guinea pigs when relatively small numbers of standard strains of *M. tuberculosis* were suspended in hog gastric mucin and injected subcutaneously. Milzer and Levine[9] described enhancement of infection in pigmented mice injected intraperitoneally with approximately 500,000 organisms of strain H37Rv suspended in hog gastric mucin.

This report deals with experiments designed to answer the following four questions:

(*1*) How effective is the intraperitoneal inoculation with mucin in the assessment of the virulence of strains of tubercle bacilli?

(*2*) Does intraperitoneal inoculation with mucin enhance the toxicity of tubercle bacilli?

(*3*) Which organ of the infected mice produced the greatest response following intraperitoneal inoculation of relatively few virulent tubercle bacilli and mucin?

(*4*) What response was given by a large concentration of a virulent strain, as contrasted with a small concentration?

The strains of tubercle bacilli (Ravenel, H37Rv, H37Ra) were obtained from Dr. Steenken at the Trudeau Laboratories, and BCG was obtained from Phipps Institute. The bacterial inocula were prepared by removing a four-week growth on American Trudeau Society medium, emulsifying it with steel balls, and making serial dilutions in a balanced salt-casein hydrolysate diluent. Viable counts of the inocula were determined on both Norite[10] plates with penicillin and malachite green added, and on American Trudeau Society medium in tubes. The 5 per cent mucin suspension was prepared as described by Gale and Elberg.[11] Mice were inoculated by injecting 0.8 ml. of mucin on one side of the abdomen, followed immediately by 0.2 ml. of the dilutions of tubercle bacilli on the other side of the abdomen. Cultures of the minced organs of the mice were done on American Trudeau Society medium in tubes. Two strains of Swiss white mice were used: Namru and Taconic Farms (T. F.).

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[8] Mills, M. A., and Colwell, C. A.: Am. Rev. Tubere., 1939, *40*, 109.

[9] Milzer, A., and Levine, E. R.: Proc. Soc. Exper. Biol. & Med., 1948, *69*, 16.

[10] Hirsch, J. G.: Am. Rev. Tubere., 1954, *70*, 955.

[11] Gale, D., and Elberg, S. S.: J. Infect. Dis., 1952, *91*, 50.

TABLE 1

THE EFFECT OF MUCIN ON THE INTRAPERITONEAL INOCULATION OF TUBERCLE BACILLI INTO WHITE MICE

Strain	Number of Deaths		Concentration of Viable Units (Approximated) Required for Response*	
	Mucin	Saline	Mucin	Saline
Ravenel	17 of 42 (40%)	0 of 41 (0%)	10	10,000
H37Rv	9 of 41 (22%)	0 of 41 (0%)	100	100,000
BCG (Phipps)	5 of 42 (12%)	0 of 42 (0%)	1,000	10,000,000
H37Ra	3 of 42 (7%)	0 of 42 (0%)	10,000,000	10,000,000

* Response is enlargement of spleen to at least twice normal size plus either visible lesion or lesion forming adhesion.

The first experiment was designed to answer the first two questions: How effective was intraperitoneal inoculation with mucin in assessing the virulence of strains of tubercle bacilli and in enhancing the toxicity of tubercle bacilli?

Groups of 7 Namru mice each were inoculated intraperitoneally with mucin or saline and tenfold dilutions of the 4 strains of tubercle bacilli, 10^{-1} to 10^{-6}. The number of viable units inoculated was rounded to the whole nearest power of 10 for the purpose of easy comparison. The mice were observed for time of death for the first five days. The survivors were sacrificed at five- to six-day intervals, and examined for gross pathology. No cultures were done. The criterion of infectivity was enlargement of the spleen to at least twice normal size plus visible lesions in any other organ or lesions forming adhesions. The results are shown in table 1.

The infectivity of the strains inoculated intraperitoneally with mucin or saline followed their known virulence: the more virulent, the fewer organisms required. With mucin, however, fewer organisms were required than with saline, except for the strain H37Ra, in which no enhancement was observed. Deaths within two days occurred only in the animals injected with mucin and tubercle bacilli. The number of such deaths also followed both the infectivity pattern and the known virulence. Control mice injected with mucin and saline showed only one death in 14 mice within two days.

The following experiment was done to answer the third question: Which organ of the infected mice produced the greatest response?

A group of 110 Namru mice were inoculated intraperitoneally with approximately 10^4 of the H37Rv strain and mucin, and 40 mice inoculated with mucin and saline served as controls. Three mice in each group were sacrificed each day, and the spleen, liver, omentum, and lungs were removed for culture individually and for histopathologic study. In addition, heart's blood immediately after sacrifice was also cultured daily. Forty-nine deaths (34.5 per cent) were observed within two days, and two deaths (5 per cent) occurred in the controls.

The results of the cultures for tubercle bacilli are shown in figure 1. The spleen gave the greatest response to this relatively small inoculum in the twenty days of examination (42 per cent positive). The heart's blood was positive from the first to the sixth days, and sporadically thereafter. Very few positive cultures were obtained from the minced livers, despite the presence of visible lesions and demonstration of acid-fast organisms in impression smears. The last observation indicated the presence of tuberculostatic material

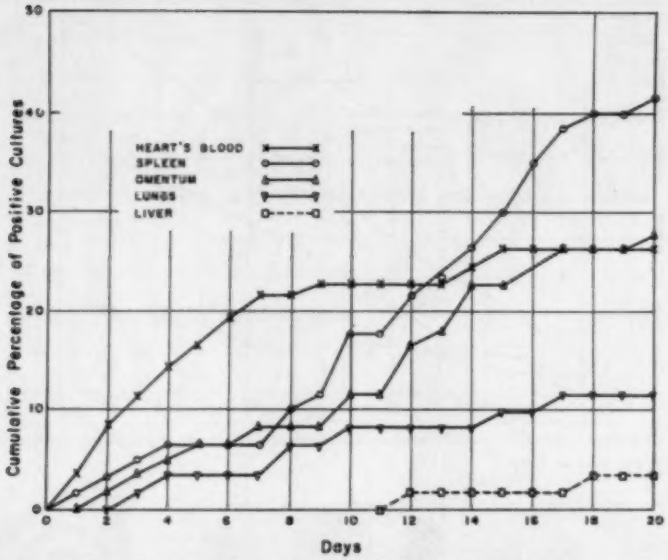

FIG. 1. Development of experimental tuberculosis as shown by cultures of individua organs positive for tubercle bacilli following intraperitoneal inoculation of mice with 10 strain H37Rv and mucin.

in infected mouse livers, a problem which is under investigation at the present time. This observation has also been made by other workers,[12, 13] but has not been published except as mentioned by Dubos.[14]

The next experiment was planned to answer the fourth question: What response was given by a large concentration of a virulent strain as contrasted to a small concentration?

Groups of 40 Taconic Farms (T. F.) mice were inoculated intraperitoneally with either 10^{-1} or 10^{-6} dilutions of H37Rv with mucin or saline, and 10 mice were injected with mucin and saline as controls. At three-day intervals, 3 mice in each group and 2 mice in the control groups were scarificed, and the liver, spleen, omentum, lungs, and kidneys were removed for culture individually and for histopathologic study. Deaths within two days occurred in 12.5 per cent of the mice which received the large inoculum and mucin and in 7.5 per cent of the mice injected with the small inoculum and mucin.

The marked difference in response between the mice injected with mucin and the large inoculum and those injected with saline, and the essentially similar response in both series with the small inoculum, are graphically demonstrated in figure 2. The actual appearance of representative half-spleens which had been fixed in formalin heightens the difference in response between the mucin and saline series given the large inoculum (figure 3). The marked enlargement of the spleen of the mucin-injected mouse at nine days following

[12] DUBOS, R. J.: Personal communication.

[13] MCDERMOTT, W.: Personal communication.

[14] DUBOS, R. J.: General discussion of papers by McDermott *et al.* and Mitchison and Selkon, Am. Rev. Tubere., 1956, *74* (Supplement, p. 116).

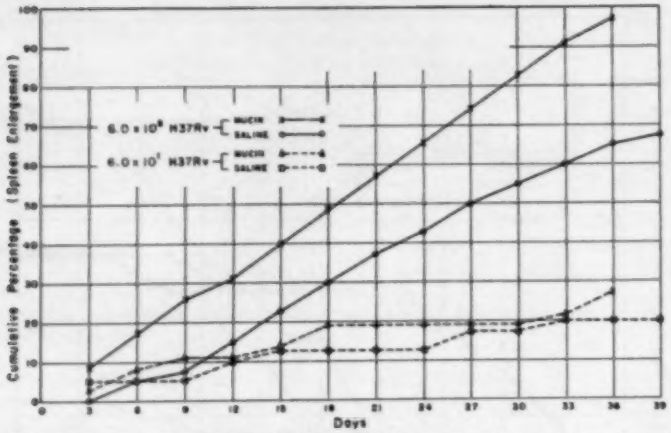

FIG. 2. Enhancement of experimental tuberculosis as shown by splenic enlargement to twice normal size or larger.

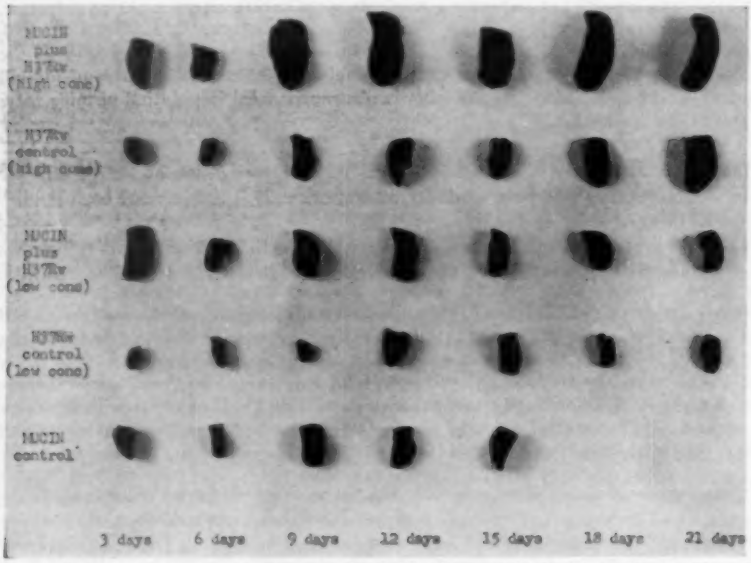

FIG. 3. Actual appearance of representative half-spleens which had been fixed in formalin.

inoculation was greater than that of the control mouse at twenty-one days. The splenic enlargement of the mice inoculated with mucin and the small inoculum, although apparently greater than that of the saline mice injected with the small inoculum, was not different from that of the mucin-injected control animals.

A contrasting pattern to the gross pathology is shown by the culture results. When the large inoculum was used (figure 4), splenic culture results of both series were similar, with

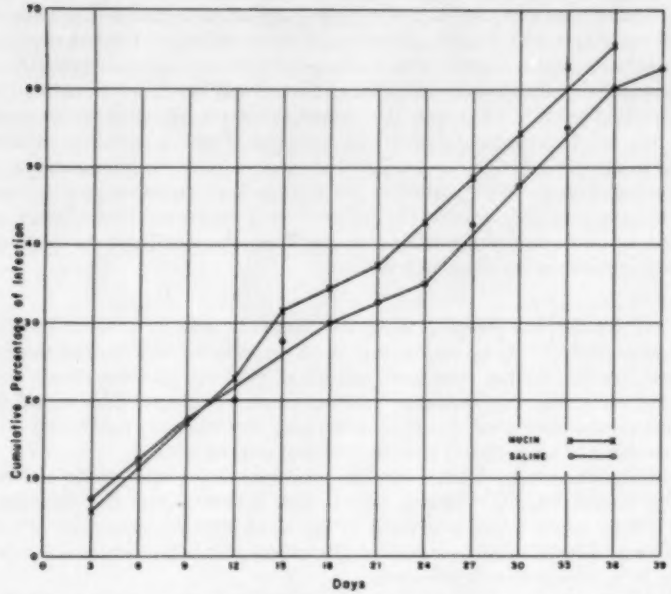

FIG. 4. The percentage of splenic cultures positive for tubercle bacilli following injection of 6.0×10^6 strain H37Rv with mucin or saline.

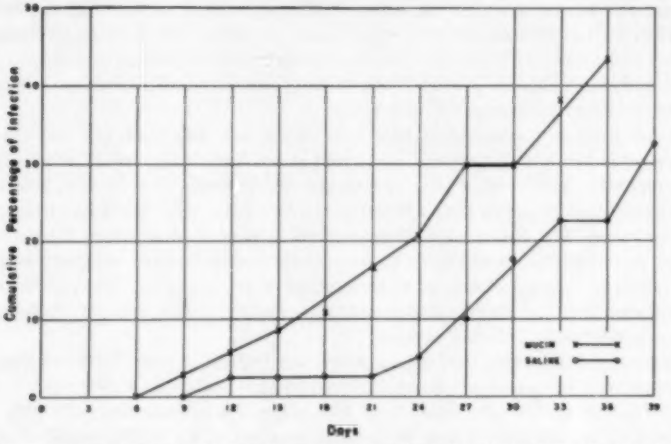

FIG. 5. The percentage of splenic cultures positive for tubercle bacilli following injection of 6.0×10^3 strain H37Rv with mucin or saline.

65 per cent positive for tubercle bacilli in the mucin animals and 60 per cent positive in the saline mice after thirty-six days. Cultures of the omentum and kidneys also showed little difference between the mucin and the saline series. However, twice as many cultures of tubercle bacilli were obtained from the lungs of the mucin mice as from the lungs of the saline series. Very few cultures positive for tubercle bacilli were isolated from the minced livers of either series, despite the presence of lesions, adhesions, or enlargement.

With the small inoculum (figure 5), the injection of mucin markedly enhanced the infection as shown by splenic culture. Twice as many cultures positive for tubercle bacilli were obtained from the mucin mice (44 per cent) as from the saline mice (22 per cent) after thirty-six days. Only a few of the cultures of the omentum, kidneys, lungs, or livers of either series were positive for tubercle bacilli. This presumably reflects the presence of only small numbers of bacilli in these tissues.

* * *

To have validity and meaning, an *in vivo* diagnostic test for tubercle bacilli should satisfy certain criteria: (*1*) It must be able to differentiate between virulent and avirulent organisms; (*2*) the response to a small number of virulent organisms should be readily detectable; (*3*) a relatively inexpensive animal which is easily available should be used; (*4*) inoculation of the animal should be done easily and relatively safely; and (*5*) results should be obtained more quickly than by the usual cultural methods.

The intraperitoneal inoculation of white mice with hog gastric mucin and standard strains of tubercle bacilli, or strains derived from patients' specimens, provides such a test. It will be shown in the next paper in this series that the inoculation of sediments derived from patients' sputums into mice with mucin gave better results in ten days than was seen by culture in four to five weeks.

Olitzki[1] and other workers have reported the fact that intraperitoneal injection of mucin enhances the virulence of microorganisms which show some degree of pathogenicity, but has no enhancing effect with nonpathogenic organisms. In the present experiments, the strain H37Ra (an avirulent variant of H37Rv) produced an identical response when injected in high concentration with either mucin or saline, but gave no pathology with a slightly lower concentration. On the other hand, the H37Rv strain was able to produce definite pathologic changes when 100 viable units were used with mucin as contrasted to the 100,000 viable units required with saline.

Whether intraperitoneal inoculation with mucin can determine the virulence of all chromogens isolated from patients' specimens is not known at present. Preliminary experiments[12] were made with a photochromogen which produced only local lesions at the site of inoculation in guinea pigs injected either subcutaneously or intraperitoneally with mucin or saline. The observations indicate that a massive progressive, tuberculosis-like infection can be produced within six to seven days in mice injected intraperitoneally with either mucin or saline and a large concentration of the organism. The photochromogen was recovered from the livers, spleens, omentum, kidneys, lungs, and various fatty tissues of the experimentally infected animals.

In summary: Mucin enhanced experimental tuberculosis in mice. With virulent strains, fewer organisms were required to produce comparable pathologic changes when injected intraperitoneally with mucin rather than with saline. No enhancement of infectivity was obtained with an avirulent strain. With small numbers of the H37Rv strain of *M. tuberculosis*, twice as many splenic cultures positive for tubercle bacilli were obtained from the mucin series as from the saline controls. With 10^6 H37Rv bacilli, marked enlargement of the spleen could be seen in the mucin mice nine days after inoculation compared with that in the saline series after only twenty-one days.

Splenic culture produced the most positive results and liver culture gave the fewest positive results in mice injected intraperitoneally with H37Rv and mucin and sacrificed daily. Heart's blood cultures were uniformly positive for tubercle bacilli for six days after inoculation, and sporadically thereafter. Deaths within forty-eight hours after inoculation were seen only in the mucin-injected animals, which was indicative of enhancement of the toxicity of tubercle bacilli.

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September 9, 1957

A RAPID MOUSE TEST FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

II. Preliminary Studies with Patients' Specimens

A more rapid diagnostic procedure to find *virulent* tubercle bacilli in patients' specimens would be of tremendous help in the treatment and control of tuberculosis. Milzer and Levine[1] in 1948 described a mouse test for the laboratory diagnosis of tuberculosis by the intraperitoneal inoculation of H37Rv or strains derived from patients, along with hog gastric mucin into pigmented mice maintained on a special diet. In 1951, Milzer, Levine, and Lewis[2] published their evaluation of such a test with 273 specimens from patients. Their conclusion was that the mouse test was inferior to either culture or guinea pig inoculation, since only 24.5 per cent of the specimens were positive for tubercle bacilli by the mouse test as against 31 per cent by guinea pig inoculation and 33 per cent by culture.

A reinvestigation of this problem was undertaken for the following reasons: (*1*) a further analysis of the figures of Milzer and associates[2] reveals that at twenty days 24.5 per cent of the specimens were positive for tubercle bacilli by the mouse test, whereas only 0.7 per cent were positive by culture and 0 per cent were positive by guinea pig inoculation; (*2*) intraperitoneal inoculation of mice is not as difficult as intravenous inoculation, or as hazardous to personnel as aerosol or intranasal instillation without special equipment; and (*3*) careful attention should be devoted to the preparation and inoculation of the mucin to ensure the most reliable and replicable results.

In the first paper in this series,[3] the enhancement effects of the intraperitoneal inoculation of standard strains of *Mycobacterium tuberculosis* with hog gastric mucin into white mice were described and discussed with relation to the formulation of a rapid mouse test. The present report is concerned with the preliminary studies to establish the technique and to compare the results obtained by the mouse test with those by culture. For this purpose, 46 sputum specimens were obtained from 16 patients who were known to be discharging tubercle bacilli, or were known to have active disease by clinical and roentgenographic evidence.

The 5 per cent mucin suspension was prepared as described by Gale and Elberg.[4] To 5.5 gm. of mucin (Wilson 1701W hog gastric granular mucin) in a Waring Blendor were added 94.0 ml. of distilled water. This was allowed to stand ten minutes for the mucin granules to absorb water. The viscid mass was blended five minutes to produce a homogeneous suspension. This suspension was autoclaved exactly fifteen minutes at 121° C. (the autoclave pressure brought down very quickly, within one to two minutes) to ensure sterility. The cooled sterile mucin suspension was adjusted to pH 7.0 by adding 10.0 ml. of sterile 0.1 N sodium hydroxide and was ready for animal inoculation. The "virulence enhancing" activity of the mucin is heat labile at neutral or alkaline pH. Fresh suspensions were prepared each day because aggregation of the granules occurs if the neutralized mucin suspension is maintained at room temperature, at 4° C., or at 37° C. for more than twenty-four hours.

Three twenty-four-hour sputum specimens were obtained daily from each of 14 patients, and two twenty-four-hour sputum specimens from 2 patients. Each of the 46 specimens

[1] Milzer, A., and Levine, E. R.: Proc. Soc. Exper. Biol. & Med., 1948, *69*, 16.
[2] Milzer, A., *et al.*: J. Lab. & Clin. Med., 1951, *37*, 803.
[3] Gale, D., *et al.*: Am. Rev. Tuberc., 1958, *77*, 1005.
[4] Gale, D., and Elberg, S. S.: J. Infect. Dis., 1952, *91*, 50.

was digested and concentrated with 4 per cent sodium hydroxide containing 1 per cent alum. The neutralized sediments were suspended as homogeneously as possible in 1.5 ml. nutrient broth by drawing up and down ten times in 1.0 ml. tuberculin syringes with twenty-four-gauge needles. One milliliter was removed for the inoculation of mice, and the remaining 0.5 ml. was used to inoculate two tubes of American Trudeau Society medium, and for acid-fast smear.

Four mice per sediment were inoculated intraperitoneally with 0.8 ml. of 5 per cent mucin on one side of the abdomen followed immediately by 0.2 ml. of sediment suspension on the other side of the abdomen. The inoculated animals were examined for death at 4-hour intervals for the first forty-eight hours. One mouse in each group was sacrificed at five days, ten days, fifteen days, and at twenty days, respectively. If 3 deaths of the 4 mice occurred within forty-eight hours, the survivor was sacrificed on the tenth day. The sacrificed mice were examined for gross pathology, with particular reference to enlargement of the spleen. The spleens and/or other organs showing lesions were removed and minced as thoroughly as possible. Two impression smears per spleen or organ were prepared for acid-fast staining. As much of the minced spleen or organ as was possible was used to prepare the impression smears in order to minimize sampling errors. The stained smears were examined for a minimum of ten minutes before they were judged to be negative for tubercle bacilli. All positive smears were verified by another examiner. A specimen was considered positive by the mouse test if *both* enlargement of the spleen was seen and acid-fast organisms were observed in impression smears of the spleen.

The cultures were read at weekly intervals, as this is the customary procedure in this laboratory and in most laboratories in which many cultures are handled.

TABLE 1

A Comparison of the Mouse Test with Culture Results in the Diagnosis of Tuberculosis

Positive Cultures Obtained	Patients		Specimens	
	Number	Per Cent	Number	Per Cent
by 5 days:				
mouse test	16 of 16	100.0	29 of 43*	67.4
culture	0 of 16	0	0 of 46	0
by 10 days:				
mouse test	16 of 16	100.0	39 of 46	84.8
culture	5 of 16	31.3	6 of 46	13.0
by 15 days:				
mouse test	16 of 16	100.0	41 of 46	90.0
culture	5 of 16	31.3	7 of 46	15.2
by 20 days:				
mouse test	16 of 16	100.0	42 of 46	91.3
culture	7 of 16	44.8	13 of 46	27.8
by 25 days:				
culture	13 of 16	81.3	21 of 46	45.6
by 32 days:				
culture	15 of 16	93.8	26 of 46	56.5
by 46 days:				
culture	16 of 16	100.0	34 of 46	74.0
by more than 46 days:				
culture	16 of 16	100.0	35 of 46	76.1
smear	14 of 16	87.5	29 of 46	63.0

* With 3 specimens, 3 of 4 mice died within 48 hours. Thus, the one survivor was sacrificed at the 10-day interval.

The results obtained by the mouse test as compared with the culture results are presented in table 1. It may be noted that: (*1*) All 16 patients were positive for tubercle bacilli by the mouse test in *five* days, whereas *forty-six* days were required for positive cultures. (*2*) The cultural results at ten days showed only 5 patients (31 per cent) positive for tubercle bacilli as contrasted to 16 patients (100 per cent) positive by the mouse test in five days. (*3*) Eighty-seven per cent of the 46 specimens were positive for tubercle bacilli by the mouse test in *ten* days as compared with 13 per cent positive by culture; 91 per cent were positive by the mouse test in *twenty* days with only 28 per cent positive by culture. (*4*) Approximately the same percentage of specimens were positive at five days by the mouse test as were obtained by culture in forty-six days. It is possible that the difference between the results of the mouse test and the culture results might have been less if the cultures had been read at more frequent intervals.

TABLE 2

THE NUMBER OF DEATHS WITHIN 48 HOURS, WITH RELATION TO THE RESULTS OBTAINED BY THE MOUSE TEST AND BY CULTURE

Correlative Data	Deaths within 48 Hours in 4 Mice per Specimen			
	0	1	2	3
Number of specimens	22	14	7	3
Number of patients	14	12	5	2*
Mouse test:				
Positive by				
5 days	14	10	5	0†
10 days	6	1	1	2†
15 days	0	2	0	0
20 days	1	0	0	0
Negative	1	1	1	1†
Culture:				
Positive by				
10 days	2	0	2	2
15 days	0	0	1	0
20 days	3	2	1	0
25 days	3	4	0	1
32 days	1	2	2	0
46 days	4	4	0	0
More than 46 days	1	0	0	0
Contaminated	7	2	0	0
Negative	1	0	1	0

* Of the 2 patients, one showed 3 of 4 deaths with 2 specimens and 2 of 4 deaths with the third specimen. The second patient only showed 3 of 4 deaths with one specimen and 0 of 4 deaths with the other 2 specimens.

† The one survivor for the 3 specimens was sacrificed only at the 10-day interval.

The cultures from 9 specimens (20 per cent) were contaminated on both tubes of medium and could not be read. All were positive for tubercle bacilli by the mouse test in twenty days, 4 in five days, 2 in ten days, 2 in fifteen days, and 1 in twenty days.

Of the 4 specimens that were negative by the mouse test, one was also negative for tubercle bacilli on culture. The spleens of the mice inoculated with the sediments of each of these specimens were enlarged to at least twice normal size, but acid-fast organisms were not visible in impression smears. In each case, the other specimens from the patient were positive by the mouse test within ten days, although cultures were either contaminated or contained only a few colonies of *M. tuberculosis* in not less than thirty-five days.

In 14 patients, at least one specimen was positive for acid-fast bacilli on microscopy. Of these 14 patients, 4 had two specimens negative by microscopy, 3 had one such negative specimen, and in 7 patients all 3 specimens were positive. Of the 29 specimens positive for acid-fast bacilli on microscopy, 10 showed "occasional" acid-fast organisms (less than 1 organism per 10 fields), 10 showed "few" organisms (1 to 2 organisms per field), and 9 showed "many" organisms (more than 5 organisms per field). The 17 specimens negative on microscopy yielded 15 positive mouse tests, 10 in five days, 3 in ten days, and one in fifteen and twenty days. The 10 specimens showing "occasional" acid-fast organisms on microscopy were all positive by the mouse test, 6 in five days, three in 10 days, and one in fifteen days. Thus, 25 of the 27 specimens (90 per cent) with relatively small numbers of organisms (showing either a negative smear, or a smear with less than one organism per 10 fields), were positive by the mouse test, with 60 per cent positive in five days.

Thirty-seven deaths were seen in forty-eight hours in the 184 mice injected with the 46 specimens (20 per cent), as compared with one death in the 12 mice injected with mucin alone (8 per cent). Necropsy examination of the test mice revealed no lesions or any other gross pathology. Some impression smears of the liver or spleen of some of these dead mice showed a few acid-fast organisms, but there was no consistent pattern to this finding.

The deaths within forty-eight hours were not used in assessing the results of the mouse test. However, a correlation of the deaths with the results obtained by the mouse test and by culture is presented in table 2. The toxicity of the tubercle bacilli in the specimens (as evidenced by death within forty-eight hours) seemed to bear little relationship to the results of the mouse test, since approximately the same number of positive cultures within five days were obtained with specimens producing 0, 1, or 2 deaths (64 per cent, 71 per cent, 71 per cent, respectively). On the other hand, some relationship could be shown with the culture results. Nine specimens producing 0 or 1 death gave cultures positive for tubercle bacilli in forty-six days or more; cultures of the specimens producing 2 deaths or more were positive within thirty-two days.

* * *

The mouse test as described in this paper is more rapidly diagnostic than either culture or smear with the 46 specimens used, since 91 per cent of the specimens were positive for tubercle bacilli by this test in twenty days, as compared with only 76 per cent positive by culture in fifty-six days, and 63 per cent on microscopy. Although these results are most promising, an evaluation of at least 1,000 specimens routinely received in the bacteriology laboratory should be done to establish this test on a firm basis. Such an evaluation is planned and will be reported.

The apparent toxicity of tubercle bacilli in patients' specimens, or of standard strains[3] as shown by deaths within forty-eight hours in mice injected intraperitoneally with mucin

and such organisms, will be discussed in detail in the third paper in this series.[6] Such toxicity has not been used in the determination of a positive result in the mouse test. It is believed[3, 5] that this manifestation of toxicity is caused by the enhancing effect of mucin on a soluble toxic material liberated by the organisms early in their *in vivo* milieu. However, the possibility cannot be excluded that the deaths within forty-eight hours may be due to: (*1*) activation and enhancement of a latent infection in the mice by the mucin plus the added adjuvant effect of the inoculated *M. tuberculosis*, or (*2*) liberation of coliforms from the gastrointestinal tract into the peritoneal cavity by inadvertent injury following the inoculation and enhancement of the coliforms by the mucin. Experiments are in progress to explore these possibilities.

In summary: A mouse test has been described by which the presence of tubercle bacilli in the sputum of all 16 patients studied was demonstrated in *five* days, whereas culture required *forty-six* days. Of 46 specimens from these patients, 91 per cent were positive for *M. tuberculosis* by the mouse test in twenty days, whereas only 76 per cent were positive by culture in fifty-six days.

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[6] GALE, D., AND JACK, A.: To be published.

ETHYL-THIO-FORMYL COMPOUND WITH ANTITUBERCULOUS ACTIVITY

Compounds containing thioethyl groups have recently[1-3] evoked considerable interest in the experimental chemotherapy of tuberculosis. These substances, although highly active in suppressing murine tuberculosis, are devoid of any *in vitro* effect on *M. tuberculosis*. Oginsky and his associates[4] have attributed the antituberculous activity of these compounds to ethyl mercaptan which is released during metabolization of these drugs. For the synthesis of peptides, Kollonitsch and co-workers[5] prepared alkyl and aralkyl-mercapto-formyl derivatives of amino acids. Some of their compounds have been studied by the present writers for their potential antituberculous activity. One of them, namely ethyl-thio-formyl-glycine ($CH_3 \cdot CH_2 \cdot S \cdot CO \cdot HN \cdot CH_2 \cdot COOH$), has been found highly effective in the treatment of murine tuberculosis.

In one series of experiments the method used in testing activity was as follows:

Groups of 12 mice were infected with the H37Rv human strain (0.1 mg. intravenously), and the drugs were administered in the diet for fourteen days, as is usually done in this Institute.[6] The diet contains about 75 per cent moisture; therefore, no extra water was given to the animals. The tests were continued until half or more of the animals in the control group were dead. Then the surviving animals were sacrificed. Activity of the drugs was evaluated on the basis of gross lung involvement, checked by histologic examination of the specimens. The score of *0* denotes no gross involvement. Nevertheless, incipient signs of tuberculosis were usually seen on histologic examination. A very advanced change, with at least two-thirds of the lung tissue involved, was scored *4*. The gradation between these extreme values was also arbitrarily chosen. The results of a typical experiment are summarized in table 1.

In another series of experiments the test was continued after 14 days of treatment until all of the animals were dead; the effect of the drug was calculated on the basis of average survival time. Animals showing no sign of tuberculosis at inspection were excluded from evaluation. One experiment of this type is demonstrated in table 2.

As shown in the tables the suppressing effect of ethyl-thio-formyl-glycine on murine tuberculosis is markedly superior to that of PAS. It is striking, however, that only a fraction of the mice treated with the formyl compound died of gross lung involvement. The animals not included in the table began to die at a time when all of the mice in the control group had already succumbed. In addition, the mean score of lung involvement in these groups is lower than that in either the control or PAS groups. These facts indicate the early death of animals which cannot be attributed to tuberculous infection alone. It is supposed that a delayed toxic effect of treatment may have played a role in the death of the animals, although administration of the compounds was interrupted on the fifteenth day.

In accordance with the negative result of treatment with benzyl-thio-formyl-glutamic acid, no beneficial effect was observed following the use of methyl-thio-formyl-glycine, butyl-thio-formyl-DL-alanine or with benzyl-thio-formyl-glycine when they were administered at a 0.5 per cent concentration in the diet of infected animals.

[1] DEL PIANTO, E.: Ricerca sci., 1950, *20*, 83.
[2] SOLOTOROVSKY, M., *et al.*: Am. Rev. Tuberc., 1954, *70*, 806.
[3] SOLOTOROVSKY, M., *et al.*: Am. Rev. Tuberc., 1956, *74*, 59.
[4] OGINSKY, E., *et al.*: Am. Rev. Tuberc., 1956, *74*, 78.
[5] KOLLONITSCH, J., *et al.*: Chem. Ber. 1956, *89*, 2293.
[6] KOCZKA, I., *et al.*: Orv. Hetil., 1951, *No. 16*, 498.

TABLE 1

ANTITUBERCULOUS ACTIVITY OF SOME COMPOUNDS SHOWN BY GROSS LUNG SCORE

Compound (0.5% in the diet)	Mean Gross Lung Score*
Ethyl-thio-formyl-glycine	0.2
Benzyl-thio-formyl-glycine	3.9
PAS	1.3
None (control)	3.7

* The experiment was terminated on the twenty-second day.

TABLE 2

AVERAGE SURVIVAL TIME IN MOUSE GROUPS OF 22 ANIMALS EACH

Compound	Concentration	Number of Mice Which Died*	Mean Score of Lung at Death	Average Survival Time
	per cent			*days*
Ethyl-thio-formyl-glycine	0.5	12	2.3	42.3
	0.25	17	2.1	40.0
PAS	0.5	22	3.4	29.5
	0.25	22	3.3	22.7
None (control)		22	3.7	18.0

* Animals with only macroscopic signs of tuberculosis.

Ethyl-thio-formyl-glycine displayed no bacteriostatic effect on strains of *M. tuberculosis* under *in vitro* conditions either in deep (Dubos Tween® 80 medium) or in surface (Kirchner medium) cultures.

The present observations serve as additional data to confirm the antituberculous activity of thioethyl compounds. As far as is known, no thioethyl compounds in which this group is substituted by formyl linkage to amino acids have so far been investigated.

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October 4, 1957

THE PREPARATION OF TUBERCULOUS SPUTUM FOR MEMBRANE FILTER FILTRATION[1]

Those who have tried the membrane filter in tuberculosis diagnostic culture work[2-4] are in agreement that, if the filter is to become a useful cultural aid, better methods than those now currently used are required for the concentration and decontamination of sputum before filtration. When material submitted for examination contains few tubercle bacilli these are frequently killed by the usual alkaline pre-inoculation treatment. It is for such specimens, as well as for other biologic fluids, that the membrane filter may prove most helpful. Before a project concerned with suitable medium for the growth of tubercle bacilli on the filter could be undertaken, a dependable technique for the preparation of sputum specimens for filtration was developed. With this method, adapted from several published reports, there is: rapid reduction of the viscid, tenacious sputum to a truly fluid phase with simultaneous decontamination, followed by centrifugation; and then sequestration of the tubercle bacilli from this watery phase by shaking with a hydrocarbon which is the fluid filtered for cultural use.

Materials

Enzyme: A stock solution of Pangestin[®][5] consisted of 4 gm. suspended in 100 ml. of distilled water. After high-speed centrifugation, the clear supernate was decanted and an equal amount of 12 per cent magnesium sulfate[6] was added to it. This was stored in 25-ml. amounts in deep freeze.

Decontaminants:

a. Chemical—A stock solution of a 1:500 aqueous solution of Zephiran[7] was prepared.

b. Antimicrobial drugs—Polymyxin-*b*-sulfate, a stock solution of 10,000 units per ml. in distilled water, was made up weekly.

c. Nystatin[8]—A stock solution of 1,000 units per ml. in *n*-octane[9] was used.

These three stock solutions, *a*, *b*, and *c*, were prepared weekly and stored in the cold.

Hydrocarbon-n-octane—This material is inflammable and was stored at room temperature safe from flame.

Membranes[10]—Millipore "HA" type.

Methods

Each well-mixed sputum sample of about 10 ml. was transferred to a 50-ml. centrifuge tube and to it 5 ml. of distilled water and 2 to 4 ml. of 4 per cent Pangestin (magnesium activated) were added, depending upon the viscosity; then sufficient polymyxin was added

[1] This work was supported by a generous grant from the Imperial County Tuberculosis and Health Association, Holtville, California.

[2] WAYNE, L. G.: J. Bact., 1955, *69*, 92.

[3] ROGER, F., *et al.*: Am. Rev. Tuberc., 1955, *71*, 371.

[4] HALEY, L. D., AND ARCH, R.: Am. J. Clin. Path., 1957, *27*, 117.

[5] Pangestin, manufactured by Difco Laboratories, Inc., Detroit, Michigan.

[6] MIDDLEBROOK, G., *et al.*: Am. Rev. Tuberc., 1954, *70*, 852.

[7] Alkyl-dimethyl benzyl ammonium chloride, manufactured as Zephiran by Winthrop Stearns, Inc., New York, New York.

[8] Nystatin, manufactured as Mycostatin by E. H. Squibb and Sons, New Brunswick, New Jersey.

[9] *n*-Octane from the Eastman Kodak Co., Distillation Products Div., Rochester, New York.

[10] Membranes from the Millipore Filter Corporation, Watertown, Massachusetts.

to bring the final concentration to 100 units per ml. per tube. All tubes were rubber stoppered and either shaken by machine or incubated in a water bath at 30° C. for 20 to 30 minutes with occasional shaking. An equal quantity of Zephiran chloride, 1:500, was then added to each tube and the shaking or incubation continued until tenacious specimens became truly fluid. This usually occurred in thirty to forty-five minutes, although very viscous material often required sixty minutes. All specimens were centrifuged at high speed and the supernate discarded.

About 5 ml. of distilled water and 2 to 4 ml. of *n*-octane, containing 100 units of nystatin per ml., were added to the remaining fine white precipitate in each tube at the time of the test. Vigorous shaking of the tubes followed for five minutes; then the tubes were set vertically to permit separation of the fluid phases. A membrane filter was mounted in the holder and, while partial vacuum was applied, the *n*-octane phase was skimmed off carefully *to the junction line* with a separate pipette and bulb for each specimen; the fluid, spread evenly over the surface of the membrane, was immediately drawn through by suction. If the fluid was applied carefully to the *membrane surface only*, one holder served for a number of successive membranes, instead of a separate holder for each filtration as was required with previous techniques. The vacuum was continued on each membrane until it began to whiten, then the leaf containing all the filtered tubercle organisms was quickly transferred to a properly prepared nutrient pad for growth on the membrane. In addition, the last few drops from each pipette were transferred to solid medium for control growth, and to a slide for stain.

The primary purpose of this method is to recover as many viable tubercle bacilli with the fewest possible technical procedures. This purpose immediately excluded the use of strong alkalies or acids as digestants or decontaminants, for both chemicals are known to kill many tubercle bacilli in a specimen during processing procedures.[11-12] Without repeating the minutiae of many trials, the present technique evolved through the steps hereinafter described.

From the first, reliance was placed on the enzymatic digestion of sputum to reduce the viscosity to a manageable consistency. Mycozyme®, papain,[13] trypsin,[14] and Pangestin were prepared as magnesium-activated enzymes, as described above, and tested with sputum by water bath incubation at 30° C. to determine the speed of reduction to a fluid phase.

Mycozyme was quickly eliminated because it was too slow. Trypsin, papain, and Pangestin appeared about equally efficient in reducing to a watery phase extremely viscid sputum in thirty to sixty minutes. In these and succeeding tests, the usual 4 per cent sodium hydroxide treatment of sputum was used as a control and reference agent. For many of these specimens, the 4 per cent sodium hydroxide digestion required almost two hours to reach the *truly fluid* condition. Primarily on the basis of cost and, secondarily because it is a mixture of amolytic, lipolytic, and proteolytic enzymes with a wide effective range under alkaline conditions, Pangestin was selected to continue further tests.

Decontaminants were used with Pangestin-digested specimens after centrifugation, and included 4 per cent or 0.05 per cent sodium hydroxide, 10 per cent trisodium phosphate, 10 per cent trisodium phosphate and Zephiran 1:1,000[15], and Zephiran 1:500.[16] In each case, 10 ml. of the decontaminant were added to the sputum precipitate and the

[11] Yegian, D., and Budd, V.: Am. J. Clin. Path., 1952, *22*, 456.

[12] Gray, D. F., *et al.*: Am. Rev. Tubere., 1954, *69*, 991.

[13] Mycozyme and papain, manufactured by General Biochemicals, Inc., Chagrin Falls, Ohio.

[14] Trypsin, manufactured by Difco Laboratories, Inc., Detroit, Michigan.

[15] Patterson, R. A., *et al.*: Am. Rev. Tubere., 1956, *74*, 284.

[16] Hirsch, J. G.: Am. Rev. Tubere., 1954, *70*, 312.

incubation continued for thirty minutes with occasional shaking of the specimens, followed by centrifugation. Real differences appeared among the various decontaminants.

When the alkalies were used with Pangestin-digested material, an immediate flocculation occurred—from loose to coarse floccules with the 0.05 or 4 per cent sodium hydroxide, to tough, rubbery floccules with 10 per cent trisodium phosphate and 10 per cent trisodium phosphate with Zephiran 1:1,000. This material, when filtered, quickly clogged the membranes and, since these shreds entrap many tubercle bacilli, when shaken with a hydrocarbon they completely defeated the purpose of the flotation procedure.

Zephiran when used alone, however, produced a fine white precipitate in a watery phase on centrifugation, which also clogged the filter when filtration was tried but did permit satisfactory separation of the tubercle bacilli, when shaken with *n*-octane.

As these trials were proceeding, the correlated cultural results (on Löwenstein-Jensen medium) showed that there was not a complete decontamination of specimens with any of the chemicals used. Although they are not constantly present, there are several groups of organisms that respond poorly to chemical inhibitors and occur sporadically in a sputum series, e.g., Klebsiella, Pseudomonas, and molds. Bacitracin, 15 units per ml., and polymyxin-*b*-sulfate, 100 units per ml., were tried alone and together with Zephiran. Polymyxin (nontoxic for *M. tuberculosis*) proved most effective especially when added with the enzyme so that digestion and decontamination proceeded together, with a later addition of Zephiran to complete the sterilization of saprophytes. Following a high wind in this area, mold contamination of specimens is frequent. To combat this nuisance, nystatin (a fungicide nontoxic for tubercle bacilli) is added to the *n*-octane used for flotation purposes. This fungicide is susceptible to *H* and *OH* ions and is insoluble in water, hence it was added to the shaken hydrocarbon phase *only, following* enzyme digestion and incubation.

Finally, since Zephiran is neutral and the enzymatic sputum digest weakly alkaline the resulting digest precipitate need not be further neutralized before shaking with the hydrocarbon. The results of this digestion-inhibitor procedure in controlling decontamination, as proved on later culture, are shown in table 1 in comparison with the results of similar treatment of the divided sputum specimens with conventional 4 per cent sodium hydroxide. Data on the membrane filter cultures following this technique are reserved for a later paper.

TABLE 1

COMPARATIVE EFFICIENCY OF TWO MODES OF SPUTUM DIGESTION-DECONTAMINATION FOR DIAGNOSIS OF M. TUBERCULOSIS BY SMEAR AND CULTURE

Digestion-Decontamination	Duplicate Specimens	Smears		Cultures			
		Positive	Negative	Löwenstein-Jensen Medium			
				Positive	Negative	Bacteria	Molds
4% Sodium hydroxide*.....	143	25 (18%)	117 (82%)	18 (12%)	125 (88%)	15	9
4% Pangestin,† Zephiran, antimicrobial drugs.....	143	33 (23%)	110 (77%)	33 (23%)	110 (77%)	6	0

* 4% sodium hydroxide digestion-decontamination, 45 to 60 minutes; neutralized with hydrochloric acid, sediment cultured.

† 4% pangestin-polymyxin digestion-decontamination, 30 to 30 minutes; Zephiran 1:500 decontamination for 15 minutes—total of 45 to 60 minutes; resuspended in water and *n*-octane, shaken, *n*-octane interface cultured.

The use of hydrocarbon for the separation of tubercle bacilli from a water phase is not new. Lange and Nitsche[17] in 1909 were probably the first to try this method with ligroin. As part of a larger problem concerned with the movement of bacteria in the living body, Mudd and Mudd[18, 19] studied the phenomenon of the abrupt passage of acid-fast organisms from oil to a water phase as they reached the interface. They concluded that ordinary bacteria that contain many polar groups do not readily pass the barrier, but that the many nonpolar groups that characterize the fatty substance of the acid-fast bacillus surface prevent wetting by water. These nonpolar groups did permit wetting—and hence transfer—by such hydrocarbons as carbon tetrachloride, cyclohexanol, chloroform, xylol, and petroleum. Later both Pottenger[20] and Smith[21, 22] used xylol satisfactorily as a separating agent in preparing sputum for culture. While this hydrocarbon does not dissolve the membrane when filtration is tried (as do most of the other hydrocarbons mentioned), it does render the membrane transparent which, when dry, does not uniformly permit the back diffusion of liquid medium for the impinged organisms. The much lighter, more volatile hydrocarbon, *n*-octane, not only sequesters the tubercle bacilli satisfactorily from the water phase by shaking, but does not affect the membrane when filtered through it. The creamy *n*-octane fluid may be used to inoculate either solid medium or membranes by filtration; it evaporates readily and leaves no inhibitory residue.

As this portion of the work was being concluded, Palen and colleagues[23] reported the similar use of pentane[24] in separating tubercle bacilli in sputum after pepsin digestion. These workers found the pentane to be nontoxic for *M. tuberculosis*. No mention was made of the membrane filter use. Pentane was therefore compared with *n*-octane for its effect on the membrane during the filtration, as a sequestering agent and for rate of evaporation.

Pentane has no observable effect upon the membrane during filtration, and adequate back diffusion of nutrient medium results. It evaporates from the membrane readily. Either *n*-octane or pentane may be recommended, therefore, for use in the manner described to separate and transport tubercle bacilli from sputum sediment to the membrane surface for filtration and culture.

In summary: A technique is described by which tubercle bacilli in a sputum sample, prepared by enzyme (Pangestin) digestion and simultaneous decontamination (Zephiran, polymyxin) are sequestered into a hydrocarbon (*n*-octane) phase by vigorous shaking. The *n*-octane phase, freed of sputum debris and most of the saprophytic organisms, is filtered in turn through a membrane filter (or filters) to collect the tubercle bacilli for culture on the membrane surface.

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October 15, 1957

[17] LANGE, L., AND NITSCHE, P.: Deutsche med. Wchnschr., 1909, *35*, 435.
[18] MUDD, S., AND MUDD, E. B. H.: J. Exper. Med., 1924, *40*, 647.
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[20] POTTENGER, J. E.: Am. Rev. Tubere., 1931, *24*, 583.
[21] SMITH, C. R.: Am. Rev. Tubere., 1938, *37*, 525.
[22] SMITH, C. R.: Am. Rev. Tubere., 1938, *38*, 57.
[23] PALEN, M. I., *et al.*: Am. Rev. Tubere., 1957, *75*, 148.
[24] Pentane, manufactured by Eastman Kodak Co., Distillation Products Div., Rochester, New York.

THE DETERMINATION OF SERUM CONCENTRATIONS OF AMPHOTERICIN B IN MAN

A Preliminary Report

A clinical evaluation of amphotericin B (Fungizone[1]) as an antimycotic agent at Fitzsimons Army Hospital necessitated an investigation of certain aspects of the pharmacology of this drug. The present report presents the results of a preliminary study on the concentrations of the drug attained and maintained in man after intramuscular and intravenous administration. The bioassay technique for determining drug concentrations and those properties of the drug affecting the assay are included.

To be of greatest value the ideal bioassay procedure should be rapid and reproducible, should detect low concentrations of the drug being studied, and the materials used or the techniques involved should not be too complex. With these requirements in view, various media, incubation periods, alterations of pH, variations in inoculum size, and drug stability studies were designed. The method described in this report has met the above criteria and utilizes materials readily available in most laboratories.

Single-strength nutrient broth with 1 per cent added dextrose, and a final pH of 7.0, was sterilized and 1 ml. was placed in each of a series of tubes. Amphotericin B was further diluted to 10 γ per ml. in a sterile solution of 50 per cent serum and saline. The controls and test serum were set up in duplicate as follows: One milliliter of the 10 γ per ml. amphotericin B in serum-saline and/or test serum was added to the first tube of a series of ten tubes containing 1 ml. of medium per tube, mixed, and 1 ml. was serially transferred through the ten tubes. A 24-hour agar slant culture of *Candida albicans* (No. 1539)[1] was suspended in saline diluted to 50 per cent T (Lumetron). One drop of this suspension was added to each of the ten tubes, using a 5 ml. pipette.

The tubes were then incubated at 37° C. and read at 24 hours. The quantity of drug present in the unknown serum was calculated by multiplying the minimal inhibitory concentration of the test organism by the lowest dilution of serum macroscopically showing the absence of growth. The results are expressed as a range and in micrograms per milliliter of serum.

The developmental stages of the bioassay technique necessitated ascertaining the stability of the drug. Sterilization, effect of temperature, and pH were investigated.

Littman[2] stated that amphotericin B was not filterable through a sintered glass filter. In testing for the presence of the drug added to urine, it was found that after Seitz filtration no amphotericin B could be detected. Seitz filtration of a variety of solutions of amphotericin B revealed that, in filtrates of either serum or serum-saline, the loss due to filtration was approximately 50 per cent. Thus serum appears to have a protective effect, as yet unexplained, on amphotericin B subjected to Seitz filtration.

The effect of temperature on amphotericin B activity was studied and it was found that the higher temperatures may change the measurable concentration of the drug Serum and working solutions containing amphotericin B stored at 4° C. and 0° C. showed no loss of activity after a six-week period. Storage at 37° C., however, resulted in a loss of activity that was detectable within two days and progressed during the two weeks of

[1] Generously supplied by the Squibb Institute for Medical Research, New Brunswick, New Jersey.

[2] LITTMAN, M. L.: Abstract presented at the Conference on Coccidioidomycosis, February 11–13, 1957, Phoenix, Arizona.

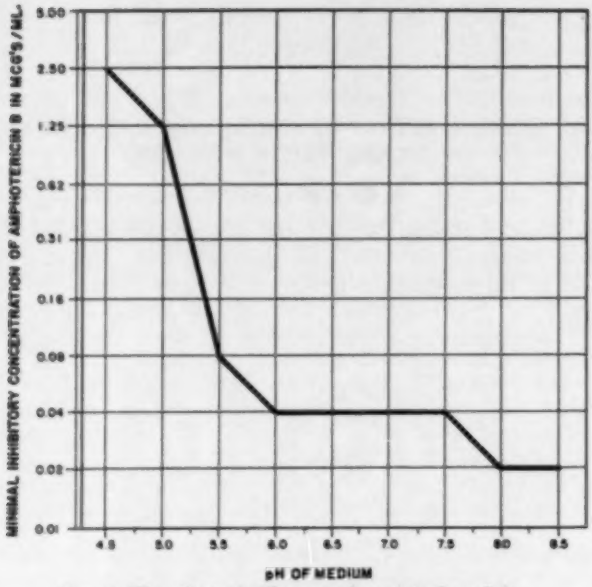

FIG. 1. The effect of pH on amphotericin B activity.

TABLE 1

BIOASSAY OF AMPHOTERICIN B

Patient	Drug per Patient	Drug and Route of Administration	Total Drug Received	Bioassay of Amphotericin B (γ/mL.) Hour Specimen Collected								
				During Infusion of Drug			After Infusion or Injection					
				0	3	6	1	2	3	6	12	24
	mg./kg.		mg.									
1	1.0	Intramuscular suspension with procaine	50				0†	0	0	0	0	0
1A*	1.0		50				0	0	0	0	0	0
2	1.0		50				0	0	0	0	0	0
3	1.0	Intravenous water-soluble drug	60	0	0.1-0.2	0.2-0.4	0.2-0.4		0.1-0.2	0.1-0.2		
3A*	1.0		60		0.1-0.2	0.2-0.4	0.2-0.4		0.1-0.2	0.1-0.2		
4	1.0		60		0.1-0.2	0.2-0.4	0.2-0.4		0.2-0.4	0.1-0.2		
5	0.5		25				0.1-0.2		0.1-0.2	0.1-0.2	0.05-0.1	0.05-0.1
6	0.75		30				0.2-0.4		0.2-0.4	0.2-0.4	0.1-0.2	0.1-0.2
7	0.75		35				0.2-0.4		0.2-0.4	0.2-0.4	0.1-0.2	0.1-0.2
8	0.75		45				0.2-0.4		0.2-0.4	0.2-0.4	0.2-0.4	0.1-0.2
9	0.25	Intravenous drug suspension	15				0		0	0	0	0
10	0.75		45				0.05-0.1		0.05-0.1	0	0	0

* 1A and 3A were repeats on patients 1 and 3.

† The sensitivity limit of the test is 0.05 γ. Results below this level are reported as 0.

storage. This loss of activity at 37° C. makes it imperative that all specimens not immediately assayed be frozen or stored at 4° C. Aqueous solutions of amphotericin B containing 100 γ per ml. lost all activity after fifteen minutes at 250° F.

The effect of pH on the antifungal activity of the drug was determined and a direct correlation observed. Increasing the pH of the medium increased the apparent activity of the drug and thus an increased drug-susceptibility was observed. The results are presented in figure 1. It should be noted that within the pH range of 6.0 to approximately 7.5 the activity is uniform; however, above this range increased activity was apparent.

Ten patients with a diagnosis of systemic fungal disease received amphotericin B. Various dosages of drug were administered and bleedings were obtained during and up to twenty-four hours following the four to six hour infusion. Three patients received intramuscular injections of amphotericin B in suspension with 1 per cent procaine. The sera from these subjects contained no detectable drug, i.e., less than 0.05 γ per ml., at any hour after injection. Two patients received intravenous infusions of amphotericin B in suspension, and the remaining 6 patients received intravenous infusions of water-soluble drug. The bioassay results for amphotericin B are presented in table 1.

Inability to detect drug concentrations in the serum of patients receiving intramuscular injections of the drug, in combination with procaine, led to a series of *in vitro* experiments to determine the antagonistic effect of procaine on drug action. The addition of procaine in amounts up to 3,300 γ per ml. to amphotericin B had no measurable effect on the recoverable amounts of amphotericin B or the assay organism.

In summary: A simple reproducible technique for the bioassay of amphotericin B in human serum is presented. The effect of filtration, pH, and temperature on amphotericin B solutions has been studied.

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December 26, 1957

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THE EFFECTS OF VARIOUS METHODS OF EXTRACTION ON THE STAPHYLOCOCCAL INFECTION-ENHANCING PROPERTIES OF TUBERCLE BACILLI

In previous publications,[1, 2] the present writers have reported that an infection of mice with staphylococci or typhoid bacilli, which by itself was not lethal, became lethal if the animals were injected simultaneously with heat-killed mycobacteria or with mycobacteria which had been extracted with alcohol-ether and chloroform and had been subjected to hydrolysis with alkali and digestion with trypsin. The analysis of the amino acid, sugar, nitrogen, and phosphorus content of these cell residues did not permit any conclusion as to the nature of the constituents responsible for the enhancing effect of these materials on the heterologous infections.

Similar observations have been reported by Dubos and Schaedler.[3, 4]

The experiments to be reported here were designed to obtain further insight into the nature of the infection-enhancing factors by studying the effects of mycobacteria submitted to other extraction procedures.

Two preparations of the polysaccharide II fraction isolated from unheated culture filtrates of tubercle bacilli were also studied for their infection-enhancing properties.

Cultures of an attenuated strain of *M. tuberculosis* BCG (Phipps) and of a virulent bovine strain of *M. tuberculosis* (Vallée) were used. The bacteria were cultivated in Roux bottles on modified Wong-Weinzirl medium (2 per cent glycerol and 0.5 per cent glucose). Two- to three-week-old cultures were washed three times with neutral isotonic phosphate-buffered saline and were collected on filter paper. The bacteria were sterilized in flowing steam for thirty minutes, and the bacterial mass was extracted at first with acetone, then with alcohol-ether (1:1), and finally with chloroform. The extractions with each solvent were repeated until no residue could be found in the extract after evaporation. Then the bacteria were dried and extracted in a solution of phenol (8 parts of 88 per cent phenol and 1 part of distilled water), at first for five hours at 60° C. and then for twenty-four to forty-eight hours at 40° C. The extraction procedure was repeated twice. The bacteria were separated by centrifugation, washed with 95 per cent alcohol until no odor of phenol could be detected, and dried at 37° C. Then they were suspended in water adjusted to pH 7.6 to 7.8 and submitted to digestion with trypsin for a period of five to six days at 37° C. under the protection of a layer of toluene. Finally, they were separated by centrifugation, washed with water, dehydrated with alcohol and alcohol-ether, and dried at 37° C.

For investigation of the infection-enhancing properties of the untreated and of the extracted bacteria, samples were taken before and after each step of extraction. Suspensions of living and steam-killed bacteria were prepared by shaking the bacteria in phosphate-buffered saline with glass beads. The suspensions were made to contain 250 mg. wet weight per ml. Suspensions of bacteria obtained after the various extraction procedures were made by careful grinding of the bacteria in a mortar and adding the saline solution dropwise in order to obtain a homogeneous suspension. These suspensions were adjusted to a concentration of 25 mg. dry weight per ml.

The tuberculo-polysaccharide II used in these experiments was obtained from unheated filtrates of cultures of human tubercle bacilli grown on Sauton medium for eight to ten weeks. This fraction was isolated according to the method described by Seibert.[5] Prepara-

[1] Rzucidlo, L., *et al.*: Bull. Acad. polonaise d. Sc., 1957, *5*, 125 (Serie des sciences biologiques).

[2] Rzucidlo, L., *et al.*: Bull. Acad. polonaise d. Sc., (in press).

[3] Dubos, R. J., and Schaedler, R. W.: J. Exper. Med., 1957, *106*, 703.

[4] Schaedler, R. W., and Dubos, R. J.: J. Exper. Med., 1957, *106*, 719.

[5] Seibert, F. B.: Am. Rev. Tubere., 1949, *59*, 86.

TABLE 1

THE INFLUENCE OF LIVING, HEAT-KILLED, AND EXTRACTED BCG BACILLI ON STAPHYLOCOCCAL INFECTION IN MICE

Experiment Number	Intraperitoneal Injection of:	Number of Mice That Died on Day—						Total Number*
		1	2	3	4	5	6	
1	2 weeks old, living BCG† + staphylococci	8 of 10	0 of 10	0 of 10	0 of 10	0 of 10	0 of 10	8 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5
2	3 weeks old, living BCG† + staphylococci	2 of 10	1 of 10	2 of 10	0 of 10	0 of 10	0 of 10	5 of 10
	Without staphylococci	1 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	1 of 5
3	3 weeks old, killed BCG† + staphylococci	17 of 20	1 of 20	0 of 20	0 of 20	0 of 20	0 of 20	18 of 20
	Without staphylococci	0 of 10	0 of 10	0 of 10	0 of 10	0 of 10	0 of 10	0 of 10
4	3 weeks old, killed BCG‡ extracted with acetone + staphylococci	6 of 10	1 of 10	0 of 10	0 of 10	0 of 10	0 of 10	7 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5
5	3 weeks old, killed BCG‡ extracted with acetone and $CHCl_3$ + staphylococci	2 of 10	1 of 10	1 of 10	1 of 10	1 of 10	1 of 10	5 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5
6	3 weeks old, killed BCG‡ extracted with acetone and $CHCl_3$ + phenol-water and digestion with trypsin + staphylococci	1 of 10	0 of 10	1 of 10	0 of 10	0 of 10	0 of 10	2 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5

* Number of mice dead/number of mice infected.
† 50 mg. wet weight.
‡ 5 mg. dry weight.

tions from two different strains were used. One was obtained from strain C^6 and gave a very viscous and opalescent solution; the other, from the strain H37Rv, gave a clearer and non-viscous solution. Solutions were prepared containing 25 mg. per ml. of saline.

For the infection of mice a freshly isolated, mannitol-fermenting, coagulase-positive strain of *Micrococcus pyogenes* var. *aureus* was used. The bacteria were cultivated for twenty-four hours at 37° C. on nutrient agar and suspended in phosphate-buffered saline to give an optical density of 0.2 (λ 570).

Mice of the CF_1 strain were injected intraperitoneally with 0.4 ml. of a suspension of equal volumes of staphylococcal and mycobacterial suspensions or of polysaccharide solu-

[6] This strain is a virulent strain of human origin which was obtained from the State Institute of Hygiene in Warsaw (Poland) and came originally from the Statens Serum Institute in Copenhagen (Denmark).

TABLE 2

THE INFLUENCE OF HEAT-KILLED AND EXTRACTED BACILLI OF THE VALLÉE STRAIN OF STAPHYLOCOCCAL INFECTION IN MICE

Experiment Number	Intraperitoneal Injection of:	Number of Mice That Died on Day—						Total Number*
		1	2	3	4	5	6	
1	Vallée,† acetone extracted + staphylococci	6 of 10	1 of 10	0 of 10	0 of 10	0 of 10	0 of 10	7 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5
2	Vallée,† acetone, alcohol-ether and $CHCl_3$ extracted + staphylococci	1 of 10	2 of 10	1 of 10	1 of 10	1 of 10	0 of 10	6 of 10
	Without staphylococci	0 of 5	0 of 5	1 of 5	0 of 5	0 of 5	0 of 5	1 of 5
3	Vallée,† acetone, alcohol-ether, $CHCl_3$, phenol-water extracted, trypsin digested + staphylococci	2 of 10	0 of 10	0 of 10	0 of 10	0 of 10	0 of 10	2 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5

* Number of mice dead/number of mice infected.

† 5 mg. dry weight of extracted bacilli prepared from 3-week-old, heat-killed cultures.

TABLE 3

THE INFLUENCE OF TUBERCULO-POLYSACCHARIDES II ON STAPHYLOCOCCAL INFECTION IN MICE

Experiment Number	Intraperitoneal Injection of:	Number of Mice That Died on Day—						Total Number*
		1	2	3	4	5	6	
1	5 mg. polysaccharide II, strain *C* + staphylococci	8 of 9	0 of 9	0 of 9	0 of 9	0 of 9	0 of 9	8 of 9
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5
2	10 mg. polysaccharide II, strain H37Rv + staphylococci	0 of 10	1 of 10	1 of 10	1 of 10	0 of 10	0 of 10	3 of 10
	Without staphylococci	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5	0 of 5
	Mice Infected with Staphylococci Only							
	0.2 ml. NaCl solution + 0.2 ml. staphylococci suspension	0 of 20	0 of 20	0 of 20	0 of 2	0 of 20	0 of 20	0 of 20

* Number of mice dead/number of mice infected.

tions. Controls receiving only staphylococci or only mycobacteria or polysaccharide fractions were included in each experiment. The animals were observed for a period of seven days.

The results are reported in tables 1, 2, and 3. It may be seen that, under these experi-

mental conditions, both the injection of living and of heat-killed BCG bacteria produced a strongly enhancing effect on the staphylococcal infection. The enhancing effect of living BCG bacteria was stronger with two-week- than with three-week-old cultures. It is further evident that the various extraction procedures progressively reduced the infection-enhancing properties of the BCG bacilli. This drop of the infection-enhancing properties was most marked after extraction of the bacteria with phenol followed by digestion with trypsin. Similar results were obtained with heat-killed and extracted bacteria of the Vallée strain.

Of particular interest are the results obtained with the polysaccharide II fraction of two strains of tubercle bacilli (*C* and H37Rv). The polysaccharide fraction of strain *C* strongly enhanced the staphylococcal infection, whereas the same fraction of H37Rv, even when given in a dose twice as strong, was much less active. The fact that the solution of the polysaccharide of the strain *C* was very viscous and opalescent, whereas the solution of the polysaccharide from H37Rv gave a nonviscous solution, suggests that the difference in biologic activity may be related to the different degrees of polymerization of these two fractions.

* * *

The experiments reported here are in agreement with those reported by Dubos and Schaedler[3, 4] who have also noticed the enhancement of heterologous bacterial infections by mycobacteria. The loss of part of the enhancing property after extraction with chloroform, and especially with phenol and trypsin digestion, suggests that this property may be due to a polysaccharide or its complexes. It is known that chloroform extracts the lipopolysaccharide fraction of mycobacteria[7] and that the phenol-water solution also extracts lipopolysaccharide complexes from many bacterial species.[8-10] The observation that the highly polymerized polysaccharide II fraction was very active in enhancing the staphylococcal infection also supports this hypothesis. Further studies on the effect of the various extracts of tubercle bacilli on heterologous infections will be reported later.

In summary: The severity of a staphylococcal infection in mice was enhanced when these bacteria were injected together with living or heat-killed BCG bacteria. The enhancing effect of these bacteria on the staphylococcal infection was decreased in bacteria which had previously been extracted with lipid solvents and phenol and digested with trypsin. Heat-killed and similarly extracted bacteria of a virulent strain of *M. tuberculosis* bovine (Vallée) manifested similar properties.

Department of Research and Laboratories
National Jewish Hospital
Denver, Colorado

Ludwik T. Rzucidlo[11,12]
Tadeusz Garbinski[11,13]

January 28, 1958

[7] Asselineau, F.: Thesis, University of Paris, 1951, Librairie Arnette.
[8] Morgan, W. T. J.: Biochem., 1936, *30*, 909.
[9] Westphal, O., *et al.*: Naturforsch, 1952, *7b*, 536; 548.
[10] Westphal, O., and Luderitz, O.: Anal. Chem., 1954, *66*, 407.
[11] Visiting Fellows of the World Health Organization.
[12] Present address: Dermatology Clinic, Medical School, Warsaw, Poland.
[13] Present address: Tuberculosis Clinic, Wroclaw, Poland.

Correspondence

CULTURE OF TUBERCLE BACILLI: IN TEST TUBES OR IN BOTTLES?

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

While considerable journal space has been occupied in recent years by controversies over viability of bacilli in resected lungs, significance of atypical mycobacteria, and contagiousness of isoniazid-resistant tubercle bacilli, another controversy has been smoldering between two groups of mycobacteriologists, with practically no publicity. This is the battle of the "tube-ites" versus the "bottle-ites." The issue is quite simple: Is it better to culture tubercle bacilli in test tubes or in prescription bottles?

We wish to establish ourselves at the outset as confirmed "bottle-ites" and to state our case.

Economy: The use of prescription bottles for solid TB media has three economic advantages over the use of test tubes. A screw-capped test tube suitable for tuberculosis culture work costs between 10 and 20 cents; a one-half ounce prescription bottle costs about 2 cents, and a one-ounce bottle approximately 3 cents when purchased in quantity. A 20 by 125 mm. test tube with a conventional slant requires at least 8 ml. of medium to provide a 10 sq. cm. inoculation surface; a 12 sq. cm. surface, with uniform depth of medium, can be achieved in a one-half ounce prescription bottle with only 4 ml. of medium. Because the bottles are so inexpensive, they are discarded after one use, eliminating the expense of washing.

Performance: If medium is inspissated (or, in the case of agar, allowed to harden) in prescription bottles lying flat in a tray on a level surface, it is possible to incubate the subsequently inoculated bottles in the same position, thereby preventing the flow and accumulation of the inoculum at the bottom or side of the medium, or even on the glass, as may occur in tubes. Furthermore, by incubating the bottles in trays, one can remove and examine a whole tray of incubating cultures without the necessity of handling each culture separately. Drying of cultures on extended incubation has been no more of a problem with prescription bottles than with test tubes.

Safety: While it is never desirable to leave a culture tube lying on a desk top, rather than to replace it in a rack, occasionally this is done. A slight disturbance can send the culture tube rolling off the desk. Prescription bottles do not roll. However, occasionally a culture is dropped, even in the best-managed laboratories. A test tube dropped to a hard-surfaced floor almost invariably shatters, whereas a small prescription bottle usually will not break. As the size of the bottle increases, however, the increased weight enhances the likelihood of its breaking.

The undersigned have arrived at their conclusions after combined experience with almost a million bottles of medium inoculated for tuberculosis diagnostic and sensitivity studies. It must be pointed out that prescription bottles are also considered by us to be the container of choice for handling diagnostic fungal cultures.

The subject of test tubes versus prescription bottles has been discussed with representatives of several commercial culture medium houses. Unfortunately, to date, none have seen fit to offer the consumer an adequate choice of containers. Other bottle shapes have been offered, but none matches the advantages of the ordinary prescription bottle except, perhaps, the perfume bottles used for some fungal media. One representative suggested

that western laboratories preparing their own media favor prescription bottles, whereas those in the eastern portion of the United States prefer the test tube.

We would welcome further discussion from the "tube-ites."

Sincerely,

VETERANS ADMINISTRATION HOSPITAL
SAN FERNANDO, CALIFORNIA

LAWRENCE G. WAYNE
MILTON HUPPERT
IRVING KRASNOW

OLIVE VIEW SANATORIUM
OLIVE VIEW, CALIFORNIA

SEYMOUR FROMAN

February 10, 1958

A PROTEIN PRECIPITATED BY A MUTANT OF M. TUBERCULOSIS H37RV

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

During biochemical studies on the *M. tuberculosis* H37Rv strain, some of the cultures produced white crystals. Chemical studies on these showed them to be a protein.

The medium used for the above studies had the following composition: asparagin, 0.5 gm.; KH_2PO_4, 0.5 gm.; sodium citrate, 2.5 gm.; $MgSO_4$, 0.5 gm.; glycerol, 40 gm.; ferric ammonium citrate, 0.05 gm. pH was adjusted to 7.0 before autoclaving. The subcultures were made from the same stock cultures of *M. tuberculosis* var. *hominis* H37Rv and were maintained on a Löwenstein-Jensen slant. After about six weeks' growth, one of the flasks showed white crystals at the bottom. These could be separated from the medium by decanting the latter. Conditions necessary for this precipitation could not be discovered. There was no difference between the staining, morphology, and growth of the two strains before or after growth. The pH, nitrogen content, or amino acid composition of the medium before or after growth were the same.

All of the subcultures from the mutant (producing crystals) did not precipitate protein in any of the subcultures except one or two. In this manner the protein was produced for six consecutive subcultures, each time only one or two of the several subcultures showing crystals at the bottom. The incubation period after which the crystals appeared varied from three to twelve weeks. The size and shape of the crystals varied from powder-like to crystals as large as 1 mm. in size. They had a square shape except in one instance when they were elongated.

After the sixth subculture no crystals were precipitated even after repeated subculturing from the mutant. Fresh subcultures from the parent stock culture into twenty-four flasks showed crystals in only one flask after eight weeks.

A subculture from this into twenty flasks of fresh medium again gave protein in one flask. About 15 mg. were produced per 100 ml. of the medium. The only nitrogen source in the medium was in the form of asparagin. All attempts to obtain consistent precipitation of the protein failed.

The crystals were insoluble in water, ethyl alcohol, ether, dilute acid, or alkali. When hydrolyzed with 6N hydrochloric acid for twelve hours and chromatographed on paper, the following amino acids could be detected: arginine aspartic acid, cystine, leucine, isoleucine, valine, phenylalanine, lycine, glutamic acid, and histidine.

No other chemical studies could be carried out because of the small quantities available.

Three of the five rabbits which have been injected with tubercle bacilli gave positive

skin reactions to 0.1 ml. of 1:10,000 dilutions of the protein. The remaining two also had no reaction to OT. However, guinea pigs inoculated with H37Rv when tested with the protein failed to show any reaction although they did react to OT. Reaction in tuberculin-positive humans was negative except in one case (five were tested).

The above record of the protein is obviously scanty. It is given only to request suggestions regarding its nature and methods for its constant production and study.

VALLABHABHAI PATEL CHEST INSTITUTE
UNIVERSITY OF DELHI
DELHI, INDIA

S. CHANDRASEKHAR

November 13, 1957

Books

BLOOD TRANSFUSION IN CLINICAL MEDICINE, Second edition. By P. L. Mollison. Pp. 587, Charles C Thomas, Springfield, Illinois, 1957. Price, $9.00.

Although generically a second edition, this volume has all the attributes of a new book about a subject which has expanded vastly since Dr. Mollison's first edition of 1951. The first edition, so well received throughout the world, has been replaced by a thoroughly reorganized and lengthened work (by 131 pages). The process of sifting the older material and combining it with the greatly expanded information accumulated over the past five years in this field has been skillfully performed in a manner which retains all of the zest of the original text.

The pertinence of the material in this book dealing with the technical and theoretical problems of blood transfusion to the blood bank worker is so obvious as hardly to require mention. The ten chapters dealing with specific laboratory and clinical problems of blood transfusion cover all aspects of the subject from the drawing and preservation of blood (dealing separately with whole blood, plasma, and the various cellular co ponents) to the diagnosis and management of hemolytic disease of the newborn. Of particular interest to the technical worker are the chapters on blood groups and antibodies, revised by the author in collaboration with Marie Cutbush, as these bring together in a readily understandable form much complex information. For the clinician the varieties of transfusion hazards are thoroughly considered in a chapter apart from one dealing with the laboratory investigation of transfusion reactions.

It would be a mistake, however, to be led by its title to regard this book solely as a work on blood transfusion per se. A chapter entitled "Blood Volume" represents a masterful treatise on a subject vital to general medical knowledge and clinical investigation, the writing of which is enhanced by the author's personal participation in many of the recent advances in the understanding of the estimation and significance of plasma and erythrocyte volumes. Equally valuable are the chapters dealing with the problems of erythrocyte survival. This subject is considered not only in respect to the problems of blood transfusion but also with regard to the survival of erythrocytes in various pathologic states, and much information, both practical and theoretical, on the use of radioisotopes in clinical studies of erythrocyte survival and body fluid volume is set forth.

In short, one cannot fail to regard Dr. Mollison's second edition as one of the most significant current works on the problems of blood—its composition and measurement, its significance in the body's physiologic and pathologic states, its clinical usage and the hazards encountered therein. In this sense not only is this work a *sine qua non* for the specialized worker in blood transfusion and hematology, but also a source of essential information, as well as delightful reading, to the student, internist, pediatrician, and clinical investigator.

WILLIAM N. CHRISTENSON
New York, New York

PERSONALITY, STRESS AND TUBERCULOSIS. Edited by Phineas J. Sparer. Pp. 629, International Universities Press, Inc., New York, 1956. Price, $12.50.

The title is appropriate as an introduction to the series of papers presented by thirty-four authors, among whom may be found the names of Hans Selye, Eric Wittkower, Jay Arthur

Myers, Julius Wilson, and other investigators and practitioners in the fields of internal medicine, microbiology, endocrinology, diseases of the chest, psychiatry, psychology, and ecology.

The many disciplines represented point up the fact that much progress has been made since the time when phthisiologists, on the one hand, studied roentgenograms and litterateurs went into the tuberculous psyche on their own without benefit of medical knowledge. This collection presents a point of view and a great deal of data to support it. In tuberculosis as in other diseases "the increasingly accepted doctrine of multiple etiology better conforms with our present knowledge. . . . There is no single cause but rather an interplay of several covariables producing a disease" and the "more epidemic tuberculosis is eradicated, the more will the personality variable be accentuated as a factor in clinical tuberculosis."

In support of these views the first part of the book is dedicated to "Basic Considerations" and sets forth in summary the views of well-known authors in the field of stress research and psychosomatic medicine. The work of Thomas Holmes represents an original contribution in multidisciplinary research in which carefully studied individual cases are presented and roentgenographic changes, mood changes, and changes in the level of 17-ketosteroid excretion are correlated. This leads to the conclusion that tuberculous subjects were sensitive, anxious, rigid, and emotionally labile individuals who, compared with others, were in many ways marginal people at the time of the onset of tuberculosis. Further, alterations in 17-ketosteroid excretion paralleling changes in the course of tuberculosis suggest that adrenocortical activity plays a role in resistance to tuberculosis and that possibly the effects of life stress upon the course of tuberculosis in part may be mediated by the adrenal gland.

In the second section on "Clinical Applications" surgeons should find the paper by John Adriani on the "Psychosomatic Aspects of Chest Surgery for Pulmonary Tuberculosis" of particular interest. In the third section on "Special Problems" practical suggestions on coordination of staff services and the analysis of the problems of irregular discharge, recidivism, and self-discharge should be of particular interest to hospital administrators. Most illuminating is the paper by Harry Wilmer suggesting that, when two comparable units were studied in an endeavor to evaluate the problem of self-discharge, the inference drawn from the data suggests that the staff and not the patient sample or the hospital environment was the significant variable.

The final section, "A Psychosomatic Program of a Comprehensive Service to Hospitalized Tuberculosis Patients," is a revision of the program which was initiated in the Veterans Administration Hospital in Memphis as long ago as 1953. It puts the responsibility for the treatment of the patient squarely on the shoulders of the attending ward physician and emphasizes the necessity for a comprehensive diagnostic evaluation and mental hygiene orientation early in the course of the patient's hospitalization.

Perhaps the most remarkable part of the book is that it is written in lucid English, and the jargon ol the several specialties has been rigidly excluded. The editor, Dr. Phineas J. Sparer, who is both a member of the American College of Chest Physicians and of the American Psychiatric Association, deserves particular congratulation on this point. This is a book to be read and consulted by every physician who has any patient with tuberculosis under his care.

Beatrice B. Berle
New York, New York

EXPLORATION DE LA FONCTION RESPIRATOIRE. By H. Bours and P. Dejours. Pp. 50, Masson et Cie., Paris, France, 1957. Price, 600 fr.

In the first part of this book, Dejours indicates some of the conditions in which an evaluation of the ventilatory function is valuable and discusses the various modes of examination of the ventilatory mechanism with regard to the static anatomic aspect, namely, the volume of gas in the lungs; and the dynamic aspect, the amount of maximal ventilation.

In the second part, Bours evaluates the indications and results of the investigation of the respiratory function in a number of pathologic conditions of the lungs.

SUZANNE RENARD
New York, New York

BOOKS RECEIVED

BASIC FACTS OF MEDICAL MICROBIOLOGY. By Stewart M. Brooks. Pp. 306, W. B. Saunders Company, Philadelphia, 1958.

SELECTED EXPERIMENTS IN MEDICAL MICROBIOLOGY. By Stewart M. Brooks. Pp. 79, W. B. Saunders Company, Philadelphia, 1958.

CURRENT THERAPY 1958. Edited by Howard F. Conn. Pp. 827, W. B. Saunders Company, Philadelphia, 1958. Price, $11.00.

DER LUNGENBOECK IM RONTGENBILD. By Karl Wurm, H. Reindell, and L. Heilmeyer. Pp. 220, Georg Thieme Verlag, Stuttgart, 1958.

PRACTICAL ALLERGY. By M. Coleman Harris and Norman Shure. Pp. 471, F. A. Davis Company, Philadelphia, 1957. Price, $7.50.

CHRONIC ILLNESS IN THE UNITED STATES, Volume I: Prevention of Chronic Illness. By the Commission on Chronic Illness. Pp. 338, Harvard University Press, Cambridge, 1957. Price, $6.00.

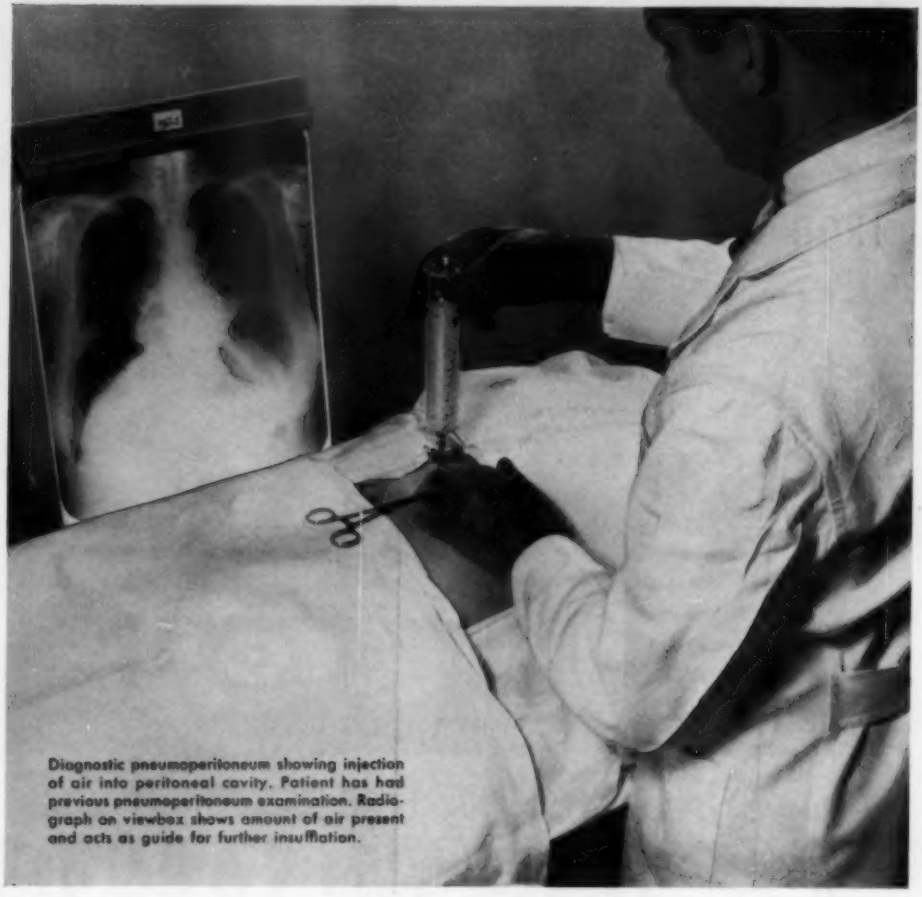
American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29
1960—Los Angeles, California—May 15–20



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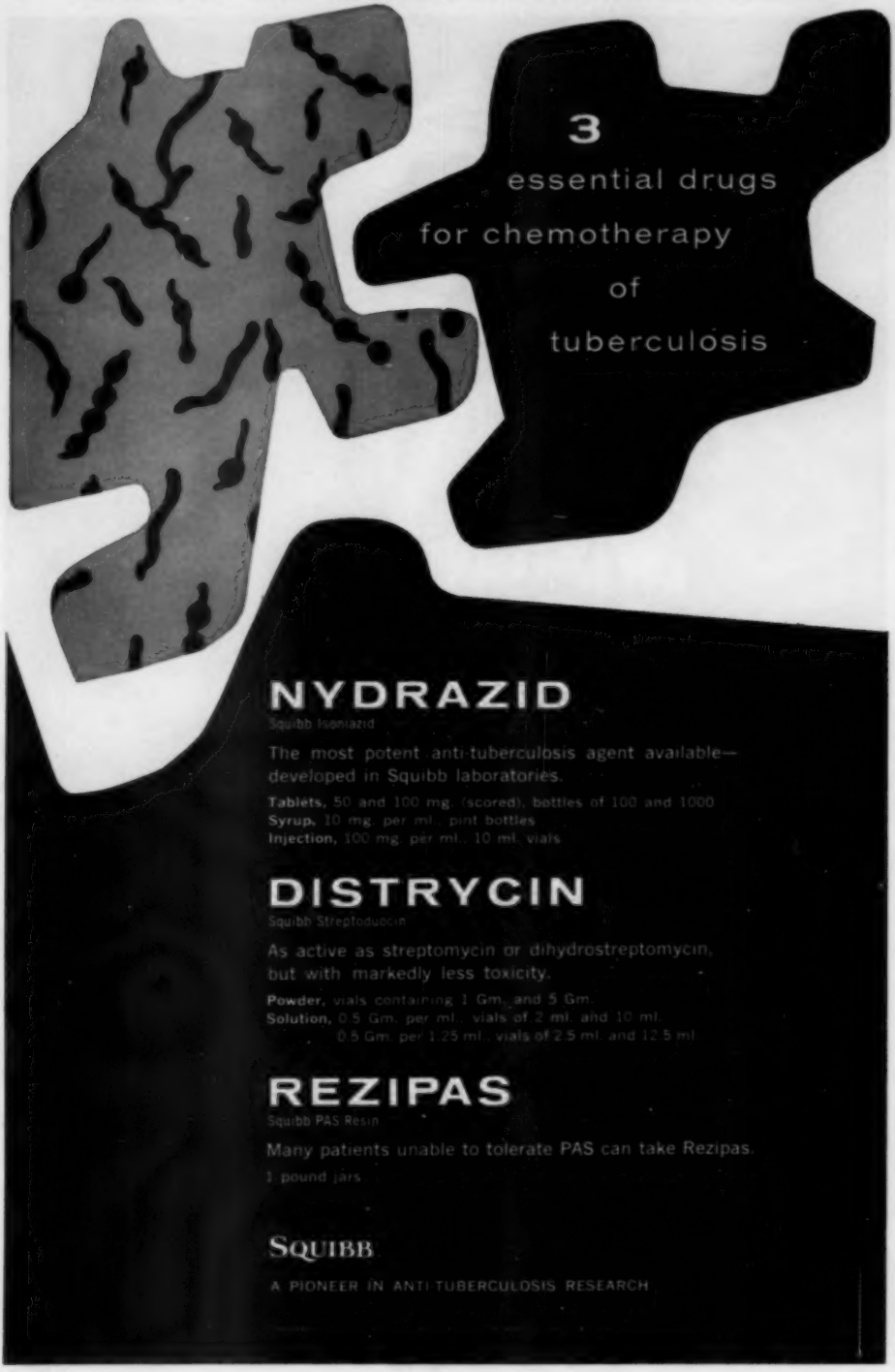
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Also available in Steraject cartridge.
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TERRAMYCIN® (Brand of oxytetracycline)

References: 1. Shubin, H., and Heiken, C. A.: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, p. 173. 2. Ross, J. D.; Horne N. W.; Grant, W. D., and Crofton, J. W.: Brit. M. J. *1*:506S (Feb. 1) 1958. 3. Kass, I.; Russell, W. F., Jr.; Heaton, A.; Miyamoto, T.; Middlebrook, G., and Dressler, S. H.: Ann. Int. Med. *47*:744 (Oct.) 1957. 4. Feldmann, F. M., Medical Director, National Tuberculosis Association: Pediatrics *21*:319 (Feb.) 1958.

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CHEST PHYSICIAN: Family, 42, seven years experience hospital management tuberculosis, in-patient and out-patient, desires permanent responsible position modern tuberculosis hospital mid-west. Address Box #152, American Review of Tuberculosis and Pulmonary Diseases, 280 Madison Avenue, New York 16, New York.

POSITION AVAILABLE: Immediate Opening, Fellow in Pediatric Pulmonary Diseases. Pediatric residency in approved hospital required. Salary $5000 per year. Affiliation with Baylor University Medical School. Send personal qualifications to Dr. Katharine Hsu, 3602 West Dallas Street, Houston 19, Texas.

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WANTED: Physician interested in Pulmonary Diseases for 170 bed state supported tuberculosis hospital; established 2 years; active surgical program, outpatient service, x-ray and laboratory; consultants in all branches. U. S. Citizenship and Alabama Medical License or eligibility therefore required. Salary $8500 to $9500 depending on qualifications. Quarters to be made available. Apply Medical Director, Sixth District Tuberculosis Hospital, 800 St. Anthony Street, Mobile, Alabama.

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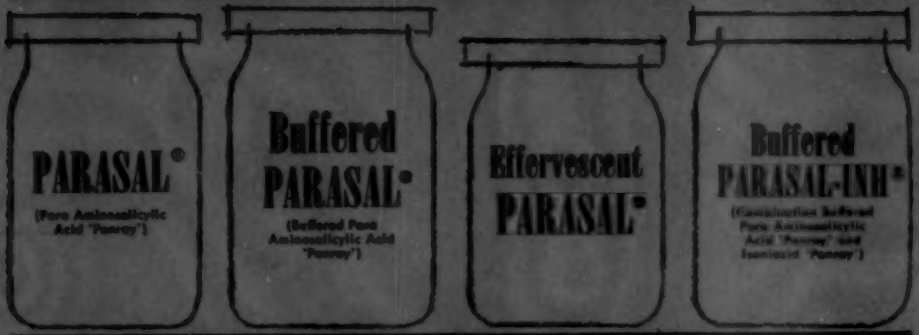

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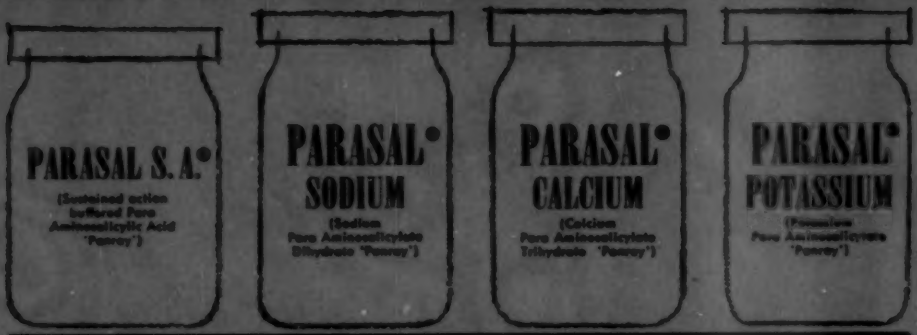
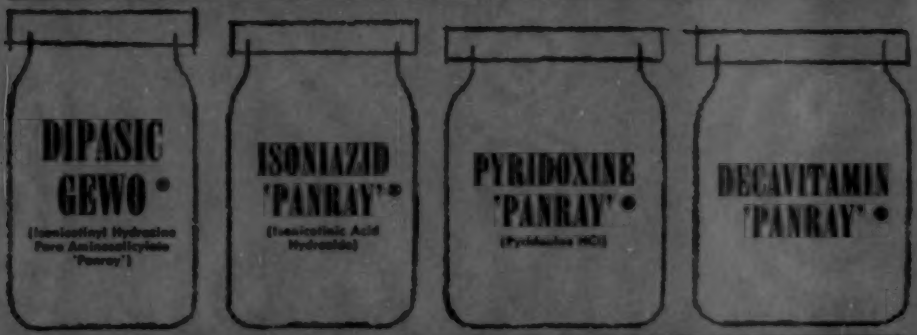
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